10/527495 Withers S

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	123	beta-glycosidase and @py<"2003"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 08:55
L2	26	L1 and mutant	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 08:49
L3	1766	beta-glycosidase or beta-glucosidase and @py<"2003"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 08:56
L4	514	L3 and mutant	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 08:57
L5	438	L4 and cellulomonas fimi	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 08:58
L6	39	l5 and Cex E127A	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:02
L7	452	(endo-acting retaining $-glycosidase) and Cellulomonas fimi	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:03
L8	167	L7 and mutant	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:09
L9	37	L8 and Cex E127A	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:03

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L10	430	beta-glycosidase and cellulomonas fimi	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:12
L11	1	L10 and (mutant enzyme CeX E127A)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	NEAR	ON	2006/05/23 09:11
L12	1201	Cex E127A	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:13
L13	72	L12 and ($glycosidase of Cellulomonas fimi)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:18
L14	2021	endo-mannanase and Cellvibrio japonicus	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:26
L15	1	L14 and Man26A	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:28
L16	337	agrobacterium and beta-glucosidase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:29
L17	123	l1 and @py<"2003"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2006/05/23 09:32
L18	0	fusion protein and (mutant of Agrobacterium beta-glucosidase,an endo-acting retaining beta-glycosidase of Cellulomonas fimi or an endo-mannanase Man26A of Cellvibrio japonicus)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	NEAR	ON	2006/05/23 09:35

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L19	0	fusion protein and (mutant of Agrobacterium $-glucosidase,an endo-acting retaining $-glycosidase of Cellulomonas fimi or an endo-mannanase Man26A of Cellvibrio japonicus)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	NEAR	ON	2006/05/23 09:37
L20	0	fusion protein and (mutant of Agrobacterium $-glucosidase,an endo-acting retaining $-glycosidase of Cellulomonas fimi or an endo-mannanase Man26A of Cellvibrio japonicus)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	NEAR	ON	2006/05/23 09:37
L21	0	fusion protein and (mutant of Agrobacterium $-glucosidase,an endo-acting retaining $-glycosidase of Cellulomonas fimi or an endo-mannanase Man26A of Cellvibrio japonicus)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:37
L22	0	mutant of Agrobacterium $-glucosidase and an endo-acting retaining $-glycosidase of Cellulomonas fimi	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:38
L23	0	mutant of Agrobacterium beta-glucosidase and an endo-acting retaining beta-glycosidase of Cellulomonas fimi	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:38
L24	0	Agrobacterium beta-glucosidase and beta-glycosidase of Cellulomonas fimi	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:39
L25	56	Agrobacterium beta-glucosidase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:47
L26	6	beta-glycosidase and cellulomonas fimi	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:53
L27	1	l26 and Cex E127A	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:54

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L28	0	endo-mannanase Man26A of Cellvibrio japonicus	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:55
L29	1	endo-mannanase Man26A	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2006/05/23 09:55

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C Mayer / D L Zechel / S P Reid / R A Warren / S G Withers, *FEBS Lett*, Jan 2000
...oligosaccharides. The 'original' glycosynthase, derived from **Agrobacterium** sp. **beta-glucosidase** (Abg) by mutating the nucleophile glutamate to alanine...glycosylated by E358S, allowing the synthesis of PNP-**beta**-LacNAc. The increased glycosylation activity of...

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D K Watt / H Ono / K Hayashi, *Biochim Biophys Acta*, Jun 1998
Agrobacterium tumefaciens **beta-glucosidase**, Cbg1 was extensively characterised and found to be a...an aryl-xylosidase. Cbg1s specificity for p-nitrophenyl **beta**-d-xylopyranoside was 73% that for p-nitrophenyl **beta**-d-glucopyranoside...

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J Zhu / S G Withers / P B Reichardt / E Treadwell / T P Clausen, *Biochem J*, Jun 1998
...natural product abundant in most members of the Salicaceae family, is a mechanism-based inactivator of **Agrobacterium** faecalis **beta-glucosidase**. Inactivation is delayed in the presence of competitive inhibitors, thereby demonstrating the requirement...

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S L Lawson / R A Warren / S G Withers, *Biochem J*, Feb 1998
...evaluation was conducted on the active-site nucleophile cysteine mutant (Glu-358-- Cys) of the retaining **beta-glucosidase** from **Agrobacterium** sp. The Glu-358-- Cys mutant was able to complete the first step (glycosylation) of the enzymic mechanism...

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Wakarchuk, W W / Greenberg, N M / Kilburn, D G / Miller, R C / Warren, R A, *Journal of Bacteriology*, Dec 2003
...DNA sequence was determined for the cloned **Agrobacterium** sp. strain ATCC 21400 **beta-glucosidase** gene, abg. High-resolution nuclease S1 protection...was homologous to a region from two other **beta-glucosidase** sequences. This region of homology contained...

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M N Namchuk / S G Withers, *Biochemistry*, Dec 1995
The role of noncovalent interactions in the catalytic mechanism of the **Agrobacterium** faecalis **beta-glucosidase** was investigated by steady-state and pre-steady state kinetic analysis of the hydrolysis of a series of monosubstituted aryl glycosides...

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☐ **8.** Identification of the acid/base catalyst in Agrobacterium faecalis beta-glucosidase by kinetic analysis of mutants.
Q Wang / D Trimbur / R Graham / R A Warren / S G Withers, *Biochemistry*, Nov 1995
The catalytic mechanism of the retaining **beta-glucosidase** (Abg) from

Agrobacterium faecalis involves a double-displacement process...rate-limiting step is deglycosylation, yielding **beta**-glucosyl azide, but had no effect on the wild...

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C Mayer / D L Jakeman / M Mah / G Karjala / L Gal / R A Warren / S G Withers, *Chem Biol*, May 2001
BACKGROUND: Oligosaccharide synthesis is becoming increasingly important to industry as diverse therapeutic roles for these molecules are discovered. The chemical synthesis of oligosaccharides on an industrial scale is often prohibitively complex and...

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T M Wrodnigg / S G Withers / A E Stütz, *Bioorg Med Chem Lett*, Apr 2001
Novel derivatives of the D-**glucosidase** inhibitor 2,5-dideoxy-2,5-imino-D-mannitol bearing lipophilic...aromatic amides attached to C-1 have been found to inhibit **beta-glucosidase** from **Agrobacterium** sp. in the nanomolar range. One of them, a coumarin...

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☐ **11.** Promoter of the rolC gene of Agrobacterium rhizogenes can be strongly regulated in glandular cell of transgenic tobacco.
Yuanlei Hu / Bojun Chen / Ting Ni / Ning Li / Zhongping Lin, *Mol Biotechnol*, Jun 2003
A 577-bp promoter segment of **Agrobacterium** rhizogenes rolC, previously known as the phloem-specific gene expression promoter, was fused to the 5' end of a reporter gene, **beta**-glucuronidase (GUS), uidA. This rolC-promoter-driven expression...

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Goyal, K. / Kim, Y.-K. / Kitaoka, M. / Hayashi, K., *Journal of Molecular Catalysis B: Enzymatic*, Nov 2001
...305-8642, Japan The **beta** -**glucosidases** of **Agrobacterium** tumefaciens and Thermotoga...Thermotoga maritima **beta** -**Glucosidase** Thermostable **Agrobacterium** tumefaciens 1 Introduction...construction of chimeric **beta** -**glucosidases** of **Agrobacterium** tumefaciens and Thermotoga...

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...natural product abundant in most members of the Salicaceae family, is a mechanism-based inactivator of **Agrobacterium** faecalis **beta-glucosidase**. Inactivation is delayed in the presence of competitive inhibitors, thereby demonstrating the requirement...

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Maeda, A. / Adachi, S. / Matsuno, R., *Biochemical Engineering Journal*, Oct 2001
...Kyoto 606-8502, Japan **Agrobacterium** tumefaciens cells...the transformation. **Agrobacterium** tumefaciens Biotransformation Cellobiose **beta -Glucosidase** d -Glucoside 3-dehydrogenase...been reported that **Agrobacterium** tumefacience cells...

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...evaluation was conducted on the active-site nucleophile cysteine mutant (Glu-358-->Cys) of the retaining **beta-glucosidase** from **Agrobacterium** sp. The Glu-358-->Cys mutant was able to complete the first step (glycosylation) of the enzymic mechanism...

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D E Trimbur / R A Warren / S G Withers, *J Biol Chem*, May 1992
The active site nucleophile of the **beta-glucosidase** of **Agrobacterium** faecalis has recently been identified by the...enzymatic mutagenesis was carried out on the **beta-glucosidase** to probe the structure of the active site region...

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J B Kempton / S G Withers, *Biochemistry*, Oct 1992
The **beta-glucosidase** from **Agrobacterium** faecalis (previously Alcaligenes faecalis) has been subjected to...slope of the leaving group-dependent portion of the Bronsted plot (**beta** 1g = -0.7) indicates a large degree of bond cleavage at the transition...

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L A Castle / K D Smith / R O Morris, *J Bacteriol*, Mar 1992
...87:3612-3618, 1990). **Agrobacterium** strains with high **beta-glucosidase** activity respond to...several fungal **beta-glucosidases**. There is little homology...and other bacterial **beta-glucosidases**, including an **Agrobacterium** cellobiase.

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S G Withers / K Rupitz / D Trimbur / R A Warren, *Biochemistry*, Oct 1992
The replacement of the active site nucleophile Glu 358 in **Agrobacterium beta-glucosidase** by Asn and Gln by site-directed mutagenesis results in essentially complete inactivation of the enzyme, while replacement by Asp...

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M C Intrieri / M Buiatti, *Mol Phylogenet Evol*, Jul 2001
...the distribution and evolution of **Agrobacterium** rhizogenes genes transferred in the...The high level of conservation of **Agrobacterium** sequences and the dependence of their...mono and multi ancient infection by **Agrobacterium**.

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Kallas, Åsa, Jan 2006
Wood and wood products are of great economical and environmental importance, both in Sweden and globally. Biotechnology can be used both for achieving raw material of improved quality and for industrial processes such as biobleaching. Despite the enormous ...
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WITHERS, Stephen, G. / JAHN, Michael / THE UNIVERSITY OF BRITISH COLUMBIA, *PATENT COOPERATION TREATY APPLICATION*, Mar 2004
Mutant glycosidases in which the amino acid in the active site that serves as the acid, base or acid/base- catalyst is converted from a carboxylic acid to some other amino acid (for example to a simple alkyl, as in alanine or glycine) can catalyze the...
Full text available at patent office. For more in-depth searching go to LexisNexis
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Yuji Hatada / Nobuhiro Takeda / Kazumichi Hirasawa / Yukari Ohta / Ron Usami / Yasuhiko Yoshida / William D Grant / (...) / Koki Horikoshi, *Extremophiles*, Dec 2005
A novel alkaline mannanase **Man26A** has been found in the culture of...mannanases reported to date. The gene **man26A** coding the enzyme was cloned from...family 26, such as the enzymes from **Cellvibrio japonicus** (37% identity), Cellulomonas fimi...
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Apr 2006
...GenBank/GenPept UniProt PDB/3D -mannanase - **Man26A** 3.2.1.78 Cellulomonas fimi AF126471 AAD42774.1 Q9XCV5 2BVT 2BVY [A] [A] -mannanase A (ManA) - **Man26A** 3.2.1.78 **Cellvibrio japonicus** (formerly Pseudomonas cellulosa) X82179 CAA57670...
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☐ **3.** ENGINEERED ENZYMES AND THEIR USE FOR SYNTHESIS OF THIOGLYCOSIDES
WITHERS, Stephen, G. / JAHN, Michael / THE UNIVERSITY OF BRITISH COLUMBIA, *PATENT COOPERATION TREATY APPLICATION*, Mar 2004
Mutant glycosidases in which the amino acid in the active site that serves as the acid, base or acid/base- catalyst is converted from a carboxylic acid to some other amino acid (for example to a simple alkyl, as in alanine or glycine) can catalyze the...
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Oct 2004
...pseudomonas cellulosa Source: **Cellvibrio japonicus**. Bacteria. Gene: **man26a**. Expressed in: escherichia...manno-oligosaccharides Source: **Cellvibrio japonicus**. Pseudomonas cellulosa...cellulosa. Bacteria. Gene: **man26a**. Expressed in: escherichia...
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Aug 2005
...2.1.78 COMPND 6 ENGINEERED: YES SOURCE MOL_ID: 1 SOURCE 2 ORGANISM_SCIENTIFIC: **CELLVIBRIO JAPONICUS** SOURCE 3 ORGANISM_COMMON: BACTERIA SOURCE 4 GENE: **MAN26A** SOURCE 5 EXPRESSION_SYSTEM: ESCHERICHIA COLI SOURCE 6 EXPRESSION_SYSTEM_COMMON...
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...COMPND 6 ENGINEERED: YES SOURCE MOL_ID: 1; SOURCE 2 ORGANISM_SCIENTIFIC: **CELLVIBRIO JAPONICUS**; SOURCE 3 ORGANISM_COMMON: BACTERIA; SOURCE 4 GENE: **MAN26A**; SOURCE 5 EXPRESSION_SYSTEM: ESCHERICHIA COLI; SOURCE 6 EXPRESSION_SYSTEM...
[http://pqs.ebi.ac.uk/pqs-doc/macmol/1j9y.mmol]
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☐ **7.** CAZy Family GH26 [95K]
Apr 2006
...Q9XCV5 2BVT 2BVY [A] [A] **Man26A** n.d. Cellulomonas fimi...4-mannanase 26B - Man26B n.d. **Cellvibrio japonicus** AY187034 AAO31762.1 Q840B9 -mannanase A (ManA) - **Man26A** 3.2.1.78 **Cellvibrio japonicus** (formerly Pseudomonas cellulosa...
[http://afmb.cnrs-mrs.fr/CAZY/fam/GH26.html]
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☐ **8.** CAZy Family CBM35 [76K]
Apr 2006
...xylanase B 3.2.1.8 **Cellvibrio japonicus** (formerly Pseudomonas...Pel10A 4.2.2.2 **Cellvibrio japonicus** (formerly Pseudomonas...Abf62A 3.2.1.55 **Cellvibrio japonicus** (formerly Pseudomonas...mannanase A (ManA) - **Man26A** 3.2.1.78 Clostridium...
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16194 Biochemistry 1995, 34, 16194-16202 Mechanism of **Agrobacterium** P-Glucosidase: Kinetic Analysis of the Role of Noncovalent EnzymeBubstrate Interactionst ...
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... of residues surrounding the active site nucleophile in **beta-glucosidase** from **Agrobacterium** faecalis - group of 3 »
DE Trimbur, RA Warren, SG Withers - Journal of Biological Chemistry, 1992 - jbc.org
... A. Region-directed Mutagenesis of Residues Surrounding the Active Site Nucleophile in @-Glucosidase from **Agrobacterium** faecaZis* ...
Cited by 22 - Web Search

Enzymatic synthesis of disaccharides using **Agrobacterium** sp. **beta-glucosidase**
H Prade, LF Mackenzie, SG Withers - Carbohydrate Research, 1997 - ingentaconnect.com
... Enzymatic synthesis of disaccharides using **Agrobacterium** sp. -glucosidase. Authors: Prade H.; Mackenzie LF; Withers SG 1. Source: Carbohydrate ...
Cited by 5 - Web Search

... and transcription analysis of the gene encoding a cellobiase from **Agrobacterium** sp. strain ATCC ... - group of 3 »
WW Wakarchuk, NM Greenberg, DG Kilburn, RC Miller ... - Journal of Bacteriology, 1988 - pubmedcentral.nih.gov
... Abstract. The DNA sequence was determined for the cloned **Agrobacterium** sp. strain ATCC 21400 **beta-glucosidase** gene, abg. High-resolution ...
Cited by 12 - Web Search

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agrobacterium AND beta-glucosidas Search

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=> s beta-glycosidase and cellulomonas fimi
L1 14 BETA-GLYCOSIDASE AND CELLULOMONAS FIMI

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=> s l1 and mutant
L2            9 L1 AND MUTANT

=> s l2 and Cex E127A
L3            0 L2 AND CEX E127A

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                ANSWERS '3-6' FROM FILE CAPLUS
                ANSWER '7' FROM FILE SCISEARCH

=> d his l4

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=> d l4 ibib abs total

L4   ANSWER 1 OF 7  BIOSIS  COPYRIGHT (c) 2006 The Thomson Corporation  on STN
     DUPLICATE 2
ACCESSION NUMBER:   1998:432217  BIOSIS
DOCUMENT NUMBER:    PREV199800432217
TITLE:              Insights into transition state stabilization of the
                    beta-1,4-glycosidase Cex by covalent intermediate
                    accumulation in active site mutants.
AUTHOR(S):          Notenboom, Valerie; Birsan, Camelia; Nitz, Mark; Rose,
                    David R. [Reprint author]; Warren, R. Antony J.; Withers,
                    Stephen G.
CORPORATE SOURCE:   Dep. Med. Biophys., Univ. Toronto, Toronto, ON M5G 2M9,
                    Canada
SOURCE:             Nature Structural Biology, (Sept., 1998) Vol. 5, No. 9, pp.
                    812-818. print.
                    ISSN: 1072-8368.
DOCUMENT TYPE:      Article
LANGUAGE:           English
ENTRY DATE:         Entered STN: 7 Oct 1998
                    Last Updated on STN: 5 Nov 1998
AB   The catalytic mechanism of 'retaining' beta-glycosidases
     has been the subject of considerable interest and debate for many years.
     The visualization of a covalent glycosyl enzyme intermediate by X-ray
     crystallography was first accomplished with a saccharide substrate
     substituted with fluorine at its 2-position.  The structure implicated
     major roles for residue His 205 and for the 2-hydroxyl position of the
     proximal saccharide in binding and catalysis.  Here we have studied the
     kinetic behavior of various His 205 mutants.  One of these
     mutants, a double mutant H205N/E127A, has been used to
     stabilize a covalent glycosyl-enzyme intermediate involving an
     unsubstituted sugar, permitting crystallographic analysis of the
     interactions between its 2-hydroxyl group and the enzyme.

L4   ANSWER 2 OF 7  BIOSIS  COPYRIGHT (c) 2006 The Thomson Corporation  on STN
ACCESSION NUMBER:   2001:163164  BIOSIS
DOCUMENT NUMBER:    PREV200100163164
TITLE:              Glycosynthases: New enzymes for oligosaccharide synthesis.
AUTHOR(S):          Moracci, Marco [Reprint author]; Trincone, Antonio; Rossi,
                    Mose
CORPORATE SOURCE:   Institute of Protein Biochemistry and Enzymology, CNR, Via
                    Marconi 10, 80125, Naples, Italy
                    moracci@dafne.ibpe.na.cnr.it
SOURCE:             Journal of Molecular Catalysis B Enzymatic, (22 January,
                    2001) Vol. 11, No. 4-6, pp. 155-163. print.
```

ISSN: 1381-1177.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Apr 2001
Last Updated on STN: 15 Feb 2002

AB The mutation of putative acid/base and nucleophile of the active sites of retaining glycosyl hydrolases, together with kinetic analysis of the **mutants**, and stereochemical identification of products lead to useful information for the understanding of the reaction mechanism of these enzymes. This was the preliminary and fundamental step toward the preparation of new enzymatic activities called glycosynthases. Direct exploitation of this information has been possible, leading to the design of four new enzymes for oligosaccharides synthesis. The interest for these biocatalysts rises from the fact that the yield of the reaction can be increased and selectivity can be interpreted as key characteristic of the transfer reaction instead of a balance of hydrolytic and transferring pathways followed either by substrates and products. These new biocatalysts possess different specificities and are promising and useful tools in the construction of oligosaccharide molecules of great biological interest. This short review focused the attention on different glycosynthases obtained from four glycosyl hydrolases highlighting on the preparation and development of these new enzymes.

L4 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2003:173625 CAPLUS
DOCUMENT NUMBER: 138:205346
TITLE: Solid-phase synthesis of oligosaccharides and glycopeptides using glycosynthases
INVENTOR(S): Withers, Stephen G.; Jensen, Knud J.; Petersen, Lars; Tolberg, Jakob L.
PATENT ASSIGNEE(S): University of British Columbia, Can.
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003018605	A2	20030306	WO 2002-IB3915	20020826
WO 2003018605	A3	20040722		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003138880	A1	20030724	US 2002-228629	20020826

PRIORITY APPLN. INFO.: US 2001-314921P P 20010824

AB The present invention provides materials and methods for the solid-phase synthesis of oligosaccharides and glycopeptides. Such materials and methods include **mutant** glycosidase enzymes, or "glycosynthases", chemical-derivatized acceptor mols., and specific solid support matrixes.

L4 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:252631 CAPLUS
DOCUMENT NUMBER: 140:283382
TITLE: Engineered glycosidases with thioglycoligase activity and their use for synthesis of thioglycosides

INVENTOR(S): Withers, Stephen G.; Jahn, Michael
PATENT ASSIGNEE(S): The University of British Columbia, Can.
SOURCE: PCT Int. Appl., 36 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2004024908	A1	20040325	WO 2003-CA1398		20030912
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW					
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CA 2497881	AA	20040325	CA 2003-2497881		20030912
AU 2003266082	A1	20040430	AU 2003-266082		20030912
US 2006035342	A1	20060216	US 2005-527495		20050518
PRIORITY APPLN. INFO.:			US 2002-410502P	P	20020912
			WO 2003-CA1398	W	20030912

AB **Mutant** glycosidases in which the amino acid in the active site that serves as the acid,base or acid/base-catalyst is converted from a carboxylic acid to some other amino acid (for example to a simple alkyl, as in alanine or glycine) can catalyze the reaction of a thiosugar acceptor and an activated donor to form a thioglycoside. The 'thioglycoligases' represent a novel class of **mutant** enzymes, and represent a first aspect of the invention. Thioglycoligases can be used in accordance with the method of the invention to couple a thiosugar acceptor and an activated donor to form a thioglycoside. By selection of the donor and acceptor species, as well as the specific enzyme employed, thioglycosides of different structure and stereochem. can be obtained. The invention is exemplified by construction and use of the E171A **mutant** of Agrobacterium β-glucosidase, E127A **mutant** of **Cellulomonas fimi** β - **glycosidase**, and the E212A **mutant** of Cellvibrio japonicus endo-mannanase Man26A.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:516090 CAPLUS
DOCUMENT NUMBER: 127:132736
TITLE: Methods and compositions for synthesis of oligosaccharides using **mutant** glycosidase enzymes
INVENTOR(S): Withers, Stephen G.; Mackenzie, Lloyd; Wang, Quingping
PATENT ASSIGNEE(S): The University of British Columbia, Can.; Withers, Stephen G.; Mackenzie, Lloyd; Wang, Quingping
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9721822	A2	19970619	WO 1996-CA841	19961212

WO 9721822		A3	19970828		
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2165041		AA	19970613	CA 1995-2165041	19951212
CA 2165041		C	19970613		
US 5716812		A	19980210	US 1995-571175	19951212
CA 2238966		AA	19970619	CA 1996-2238966	19961212
AU 9711354		A1	19970703	AU 1997-11354	19961212
AU 722220		B2	20000727		
EP 870037		A2	19981014	EP 1996-942211	19961212
EP 870037		B1	20020717		
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000501607		T2	20000215	JP 1997-521572	19961212
EP 1211320		A2	20020605	EP 2001-130513	19961212
EP 1211320		A3	20050316		
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, AL				
AT 220720		E	20020815	AT 1996-942211	19961212
US 6284494		B1	20010904	US 1998-91272	19980929
US 2003100749		A1	20030529	US 2001-837711	20010417
PRIORITY APPLN. INFO.:				CA 1995-2165041	A 19951212
				US 1995-571175	A2 19951212
				EP 1996-942211	A3 19961212
				WO 1996-CA841	W 19961212
				US 1998-91272	A1 19980929

AB **Mutant** glycosidase enzymes are formed in which the normal nucleophilic amino acid within the active site has been changed to a non-nucleophilic amino acid. These enzymes cannot hydrolyze disaccharide products, but which can still form them. Using this enzyme, oligosaccharides are synthesized by preparing a mixture of an α-glycosyl fluoride and a glycoside acceptor mol., enzymically coupling the α-glycosyl fluoride to the glycoside acceptor mol. to form a glycosyl glycoside product using the **mutant** glycosidase enzyme, and recovering the glycosyl glycoside product. Particular enzymes include a **mutant** form of Agrobacterium β-glucosidase in which the normal glutamic acid residue at position 358 is replaced with an alanine residue by using oligonucleotide-directed mutagenesis. Agrobacterium E358A β-glucosidase catalyzed the reaction of α-galactosyl fluoride with p-nitrophenyl-β-D-glucoside to form p-nitrophenyl-4-O-glucopyranosyl-β-D-galactopyranoside in 84% yield. The nature of the donor moiety in some aryl glycosides shifts the reaction from β-1,4 linkages to the production of β-1,3 linkages, but still produces a good yield of product.

L4 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER:	1997:103411 CAPLUS
DOCUMENT NUMBER:	126:183018
TITLE:	Transglycosylation by wild type and **mutants** of a β-1,4-glycosidase from **Cellulomonas fimi** (Cex) for synthesis of oligosaccharides
AUTHOR(S):	Nikolova, P. V.; Duff, S.; MacLeod, A.; Haynes, C. A.
CORPORATE SOURCE:	Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE:	Annals of the New York Academy of Sciences (1996), 799(Enzyme Engineering XIII), 19-25 CODEN: ANYAA9; ISSN: 0077-8923
PUBLISHER:	New York Academy of Sciences
DOCUMENT TYPE:	Journal

LANGUAGE: English

AB The transglycosylation activities of Cex β-1,4-glycosidase have been measured in buffer and in mixed acetonitrile/aqueous solvents to determine the sensitivity of transglycosylation reactions catalyzed by this retaining glycosidase system to water activity and solvent polarity. The conformational structure pattern and thermal stability of **mutants** of Cex have been assessed by transmission CD to confirm that mutations do not result in pronounced changes in structure.

REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 7 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:882010 SCISEARCH

THE GENUINE ARTICLE: 858XQ

TITLE: Directed evolution of a glycosynthase from Agrobacterium sp increases its catalytic activity dramatically and expands its substrate repertoire

AUTHOR: Kim Y W; Lee S S; Warren R A J; Withers S G (Reprint)

CORPORATE SOURCE: Univ British Columbia, Dept Chem, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (Reprint); Univ British Columbia, Dept Chem, Vancouver, BC V6T 1Z1, Canada; Univ British Columbia, Ctr Excellence Canada, Prot Engn Network, Vancouver, BC V6T 1Z1, Canada; Univ British Columbia, Dept Microbiol & Immunol, Vancouver, BC V6T 1Z1, Canada
withers@chem.ubc.ca

COUNTRY OF AUTHOR: Canada

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (8 OCT 2004) Vol. 279, No. 41, pp. 42787-42793.
ISSN: 0021-9258.

PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 52

ENTRY DATE: Entered STN: 29 Oct 2004
Last Updated on STN: 29 Oct 2004

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The Agrobacterium sp. beta-glucosidase (Abg) is a retaining **beta-glycosidase** and its nucleophile **mutants**, termed Abg glycosynthases, catalyze the formation of glycosidic bonds using alpha-glycosyl fluorides as donor sugars and various aryl glycosides as acceptor sugars. Two rounds of random mutagenesis were performed on the best glycosynthase to date (AbgE358G), and transformants were screened using an on-plate endocellulase coupled assay. Two highly active **mutants** were obtained, 1D12 (A19T, E358G) and 2F6 (A19T, E358G, Q248R, M407V) in the first and second rounds, respectively. Relative catalytic efficiencies (k(cat)/K-m) of 1: 7: 27 were determined for AbgE358G, 1D12, and 2F6, respectively, using alpha-D-galactopyranosyl fluoride and 4-nitrophenyl beta-D-glucopyranoside as substrates. The 2F6 **mutant** is not only more efficient but also has an expanded repertoire of acceptable substrates. Analysis of a homology model structure of 2F6 indicated that the A19T and M407V mutations do not interact directly with substrates but exert their effects by changing the conformation of the active site. Much of the improvement associated with the A19T mutation seems to be caused by favorable interactions with the equatorial C2-hydroxyl group of the substrate. The alteration of torsional angles of Glu-411, Trp-412, and Trp-404, which are components of the aglycone (+1) subsite, is an expected consequence of the A19T and M407V mutations based on the homology model structure of 2F6.

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                ANSWERS '108-180' FROM FILE CAPLUS
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L3          224 DUP REM L1 (378 DUPLICATES REMOVED)
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induced, resulting in the release of mature PorA. Cleavage of the **fusion protein** was temperature- and time-dependent, and was optimal at pH 7.0 after 5 days of storage at 4 degrees C. Efficient cleavage was also dependent on the addition of a minimal amino acid sequence (Gly-Arg-Ala) to the N-terminus of the mature PorA protein. This represented a significant improvement on the large N-terminal sequences introduced by other expression systems previously used to prepare recombinant PorA, and the yields of PorA purified with the IMPACT-TWIN system were similar. Thus, the IMPACT-TWIN system provides a facile method for producing recombinant PorA and may also be useful for the production of other bacterial outer-membrane proteins for vaccine studies.

L3 ANSWER 27 OF 224 MEDLINE on STN DUPLICATE 39
ACCESSION NUMBER: 2002647938 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12406675
TITLE: Overexpression of DsbC and DsbG markedly improves soluble and functional expression of single-chain Fv antibodies in Escherichia coli.
AUTHOR: Zhang Zhong; Li Zhi-Hua; Wang Fei; Fang Min; Yin Chang-Cheng; Zhou Zhi-Yong; Lin Qing; Huang Hua-Liang
CORPORATE SOURCE: Group 102, Institute of Genetics and Developmental Biology, Academia Sinica, Beijing 100101, China.
SOURCE: Protein expression and purification, (2002 Nov) Vol. 26, No. 2, pp. 218-28.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 31 Oct 2002
Last Updated on STN: 9 May 2003
Entered Medline: 8 May 2003

AB Single-chain Fv antibodies (scFv), a group of reconstructed molecules with several disulfide bonds, are prone to aggregate as inclusion bodies, the insoluble species of natural proteins, when expressed in Escherichia coli, especially at high level. Recovery of functionally active products from inclusion bodies is onerous and ineffective. We have increased the soluble and functional scFv yields by fusing either DsbC or DsbG, two E. coli disulfide isomerases with general chaperone function, to scFvs. Compared to the totally insoluble inclusion bodies of scFvs expressed separately, more than half of each **fusion protein** DsbC-scFv or DsbG-scFv was soluble, according to SDS-PAGE analysis. The more effective solubility was obtained when the fused protein DsbG-scFv was co-expressed simultaneously with DsbC under the same promoter. Under this condition, the soluble portion of DsbG-scFv increased from about 50% to 90% measured by scanning SDS-PAGE gel. Co-expression of DsbC can change **fusion protein CBD**-scFv from totally insoluble when expressed in E. coli separately to a considerable portion of soluble **CBD**-scFv. Antigen-binding activity assay showed that scFvs retained full affinity to specific antigens. We also determined that general molecular chaperones GroEL and GroES had no effects on the solubility of scFvs when co-expressed with scFv in E. coli. We propose that the correct formation of disulfide bonds in scFvs is the crucial factor responsible for solubility of scFvs.

L3 ANSWER 28 OF 224 MEDLINE on STN DUPLICATE 42
ACCESSION NUMBER: 2001688474 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11734582
TITLE: Preparation and incorporation of probe-labeled apoA-I for fluorescence resonance energy transfer studies of rHDL.
AUTHOR: Li H H; Thomas M J; Pan W; Alexander E; Samuel M; Sorci-Thomas M G

CORPORATE SOURCE: Department of Pathology, The Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA.
CONTRACT NUMBER: HL49373 (NHLBI)
HL60079 (NHLBI)
HL64163 (NHLBI)
HL64963 (NHLBI)
SOURCE: Journal of lipid research, (2001 Dec) Vol. 42, No. 12, pp. 2084-91.
Journal code: 0376606. ISSN: 0022-2275.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 6 Dec 2001
Last Updated on STN: 16 Mar 2003
Entered Medline: 5 Mar 2002

AB Apolipoprotein A-I (apoA-I), the major constituent of HDL, plays an essential role in regulating cholesterol metabolism, acting as the physiological activator of lecithin: cholesterol acyltransferase, which converts cholesterol to cholesterol ester. Thiol-reactive fluorescent probes attached to cysteine-containing apoA-I mutants are currently being used to investigate the "LCAT active" conformation of lipid-bound apoA-I. Herein, we report new methodologies allowing rapid expression, fluorescent labeling, and recombinant HDL (rHDL) preparation for use in apoA-I in fluorescence resonance energy transfer (FRET) studies. Cysteine-containing mutant forms of human apoA-I were cloned into the pTYB12 vector containing a T7 promoter, a modified self-splicing protein element (intein), and a small affinity tag [chitin binding domain (**CBD**)]. The **fusion proteins** were expressed in Escherichia coli, isolated from cell lysates, and bound to a chitin-affinity column. Release of mature human apoA-I was initiated by the addition of DTT, which induced self-cleavage at the COOH terminus of the intein - **CBD fusion protein.** ApoA-I was further purified by Q-sepharose and then used for fluorescent probe labeling. Discoidal rHDL were then prepared with donor and/or acceptor labeled apoA-I and characterized with respect to their size, composition and ability to activate LCAT.

L3 ANSWER 29 OF 224 MEDLINE on STN DUPLICATE 44
ACCESSION NUMBER: 2001482279 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11525624
TITLE: Cloning, nucleotide sequence and expression of a hydantoinase and carbamoylase gene from Arthrobacter aurescens DSM 3745 in Escherichia coli and comparison with the corresponding genes from Arthrobacter aurescens DSM 3747.
AUTHOR: Wiese A; Wilms B; Syldatk C; Mattes R; Altenbuchner J
CORPORATE SOURCE: Institut fur Industrielle Genetik, Universitat Stuttgart, Germany.
SOURCE: Applied microbiology and biotechnology, (2001 Jun) Vol. 55, No. 6, pp. 750-7.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 30 Aug 2001
Last Updated on STN: 18 Dec 2002
Entered Medline: 17 Jan 2002

AB The genes encoding hydantoinases (hyuH1) and carbamoylases (hyuC1) from

Arthrobacter aurescens DSM 3745 and Arthrobacter aurescens DSM 3747 (hyuH2, hyuC2) were cloned in Escherichia coli and the nucleotide sequences determined. The hydantoinase genes comprised 1,377 base pairs and the carbamoylase genes 1,239 base pairs each. Both hydantoinases, as well as both carbamoylases, showed a high degree of nucleotide and amino acid sequence identity (96-98%). The hyuH and hyuC genes were expressed in E. coli under the control of the rhamnose promoter and the different specific activities obtained in E. coli crude extracts were compared to those produced by the original hosts. For purification the hyuH2 gene was expressed as a maltose-binding protein (MalE) and as an intein-chitin binding domain (**CBD**) fusion in E. coli. The expression of malE-hyuH2 resulted in the production of more soluble and active protein. With respect to temperature stability, optimal pH and optimal temperature, substrate and stereospecificity, the purified fusion enzyme exhibited properties similar to those of the wild-type enzyme.

L3 ANSWER 30 OF 224 MEDLINE on STN DUPLICATE 45
ACCESSION NUMBER: 2001438210 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11483001
TITLE: Overexpression and purification of Helicobacter pylori flavodoxin and induction of a specific antiserum in rabbits.
AUTHOR: Paul R; Bosch F U; Schafer K P
CORPORATE SOURCE: Division of Molecular Microbiology, Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland.. Ralf.Paul@Yale.edu
SOURCE: Protein expression and purification, (2001 Aug) Vol. 22, No. 3, pp. 399-405.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 29 Oct 2001
Last Updated on STN: 29 Oct 2001
Entered Medline: 25 Oct 2001

AB Flavodoxin from the gastric pathogen Helicobacter pylori has been shown to be the electron acceptor of the essential pyruvate-oxidoreductase enzyme complex and proposed to be involved in the pathogenesis of gastric MALToma. In order to obtain a sufficient amount for biochemical and structural studies, we overexpressed the protein either with a C-terminal His(6) -tag or as a **fusion protein** upstream of intein- and chitin-binding domains. With both expression systems we succeeded at purifying soluble and functional flavodoxin containing the cofactor FMN. When expressing with a His(6) -tag, we purified approximately 20 mg flavodoxin per liter of bacterial culture, while expression as an intein-**CBD fusion protein** with autocatalytic removal of the intein-**CBD** part rendered only approximately 1 mg of purified flavodoxin per liter of bacterial culture. Expressed as an intein-**CBD fusion protein**, flavodoxin copurified with a C-terminal degradation product, which was not observed for expression with a His(6) -tag. However, we were able to obtain protein crystals suited for X-ray structure determination from flavodoxin expressed as an intein-**CBD fusion protein**, but not from flavodoxin expressed with a C-terminal His(6) -tag. We further report the induction of a rabbit antiserum specific for H. pylori flavodoxin.

L3 ANSWER 31 OF 224 MEDLINE on STN DUPLICATE 47
ACCESSION NUMBER: 2001159169 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11261722

TITLE: Genetically engineered peptide fusions for improved protein partitioning in aqueous two-phase systems. Effect of fusion localization on endoglucanase I of Trichoderma reesei.
AUTHOR: Collen A; Ward M; Tjerneld F; Stalbrand H
CORPORATE SOURCE: Department of Biochemistry, Lund University, Sweden.
SOURCE: Journal of chromatography. A, (2001 Mar 2) Vol. 910, No. 2, pp. 275-84.
Journal code: 9318488. ISSN: 0021-9673.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 17 May 2001
Last Updated on STN: 17 May 2001
Entered Medline: 10 May 2001

AB Genetic engineering has been used for fusion of the peptide tag, Trp-Pro-Trp-Pro, on a protein to study the effect on partitioning in aqueous two-phase systems. As target protein for the fusions the cellulase, endoglucanase I (endo-1,4-beta-Dglucan-4-glucanohydrolase, EC 3.2.1.4, EGI, Cel7B) of Trichoderma reesei was used. For the first time a glycosylated two-domain enzyme has been utilized for addition of peptide tags to change partitioning in aqueous two-phase systems. The aim was to find an optimal fusion localization for EGI. The peptide was (1) attached to the C-terminus end of the cellulose binding domain (**CBD**), (2) inserted in the glycosylated linker region, (3) added after a truncated form of EGI lacking the **CBD** and a small part of the linker. The different constructs were expressed in the filamentous fungus T. reesei under the gpdA promoter from Aspergillus nidulans. The expression levels were between 60 and 100 mg/l. The partitioning behavior of the **fusion proteins** was studied in an aqueous two-phase model system composed of the thermoseparating ethylene oxide (EO)-propylene oxide (PO) random copolymer EO-PO (50:50) (EO50PO50) and dextran. The Trp-Pro-Trp-Pro tag was found to direct the **fusion protein** to the top EO50PO50 phase. The partition coefficient of a **fusion protein** can be predicted with an empirical correlation based on independent contributions from partitioning of unmodified protein and peptide tag in this model system. The fusion position at the end of the **CBD**, with the spacer Pro-Gly, was shown to be optimal with respect to partitioning and tag efficiency factor (TEF) was 0.87, where a fully exposed tag would have a TEF of 1.0. Hence, this position can further be utilized for fusion with longer tags. For the other constructs the TEF was only 0.43 and 0.10, for the tag fused to the truncated EGI and in the linker region of the full length EGI, respectively.

L3 ANSWER 32 OF 224 MEDLINE on STN DUPLICATE 50
ACCESSION NUMBER: 2001076551 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11078822
TITLE: Design, expression, and renaturation of a lesion-targeted recombinant epidermal growth factor-von Willebrand factor **fusion protein**: efficacy in an animal model of experimental colitis.
AUTHOR: Hall F L; Kaiser A; Liu L; Chen Z H; Hu J; Nimni M E; Beart R W Jr; Gordon E M
CORPORATE SOURCE: Division of Colon and Rectal Surgery, Keck School of Medicine of USC, Los Angeles, CA 90089, USA.
SOURCE: International journal of molecular medicine, (2000 Dec) Vol. 6, No. 6, pp. 635-43.
Journal code: 9810955. ISSN: 1107-3756.
PUB. COUNTRY: Greece
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 11 Jan 2001

AB In the present study, the mature epidermal growth factor (EGF) protein was engineered to incorporate a high affinity collagen-binding domain (**CBD**) derived from co-agulation von Willebrand factor, to specifically target EGF to colonic lesions. The **fusion protein** was expressed in an E. coli bacterial expression system, purified by metal chelate chromatography, and renatured by oxidative refolding into a soluble biologically active growth factor. The EGF-**CBD fusion protein** bound tightly to collagen matrices under conditions in which native non-targeted EGF was washed away. In biologic assays, the EGF-**CBD fusion protein** stimulated NIH3T3 cell proliferation with near wild-type biological activity. In vivo binding studies showed that the collagen-targeted EGF, but not the non-targeted EGF, accumulated at areas of exposed collagen on the luminal surface of the inflamed colon. Finally, a single colonic instillation of the collagen-targeted EGF-induced a more rapid regeneration of intestinal crypts 24 h after treatment (number of crypts = 89.2+/-8.1) compared to the non-targeted EGF (number of crypts = 52.2+/-29.8; p=0.027), and the PBS control (number of crypts = 24. 0+/-22.9; p=0.001). Taken together, these findings indicate that intracolonic delivery of collagen-targeted EGF represents a potentially effective therapeutic strategy for acute or chronic inflammatory bowel disease.

L3 ANSWER 33 OF 224 MEDLINE on STN DUPLICATE 51
ACCESSION NUMBER: 2000454833 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10918133
TITLE: Expression, immobilization, and enzymatic characterization of cellulose-binding domain-organophosphorus hydrolase fusion enzymes.
AUTHOR: Richins R D; Mulchandani A; Chen W
CORPORATE SOURCE: Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521, USA.
SOURCE: Biotechnology and bioengineering, (2000 Sep 20) Vol. 69, No. 6, pp. 591-6.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 5 Oct 2000
Last Updated on STN: 5 Oct 2000
Entered Medline: 26 Sep 2000

AB Bifunctional **fusion proteins** consisting of organophosphate hydrolase (OPH) moieties linked to a Clostridium-derived cellulose-binding domain (**CBD**) were shown to be highly effective in degrading organophosphate nerve agents, enabling purification and immobilization onto different cellulose materials in essentially a single step. Enzyme kinetics studies were performed for the **CBD**-OPH fusions using paraoxon as the substrate. The kinetics values of the unbound fusion enzymes were similar to OPH with a modest increase in K(m). Immobilization of the enzymes onto microcrystalline cellulose resulted in a further increase in the K(m) values of approximately twofold. The pH profile of the cellulose-immobilized enzymes was also only minimally affected. The **CBD**-OPH **fusion proteins** could be immobilized onto a variety of cellulose matrixes, and retained up to 85% of their original activity for 30 days. The durability of the bound

fusions increased with the amount of Avicel used, suggesting that protein/cellulose interactions may have a dramatic stabilizing effect. Repeated hydrolysis of paraoxon was achieved in an immobilized enzyme reactor with 100% degradation efficiency over 45 days. These **fusion proteins** should prove to be invaluable tools for the development of low cost, OPH-based cellulose materials for the simultaneous adsorption and degradation of stored or spilled organophosphate wastes.

L3 ANSWER 34 OF 224 MEDLINE on STN DUPLICATE 52
ACCESSION NUMBER: 2001057285 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11025543
TITLE: Alpha-amylase inhibitors selected from a combinatorial library of a cellulose binding domain scaffold.
AUTHOR: Lehtio J; Teeri T T; Nygren P A
CORPORATE SOURCE: Department of Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Proteins, (2000 Nov 15) Vol. 41, No. 3, pp. 316-22. Journal code: 8700181. ISSN: 0887-3585.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 20 Dec 2000
AB A disulfide bridge-constrained cellulose binding domain (**CBD** (WT)) derived from the cellobiohydrolase Cel7A from Trichoderma reesei has been investigated for use in scaffold engineering to obtain novel binding proteins. The gene encoding the wild-type 36 aa **CBD**(WT) domain was first inserted into a phagemid vector and shown to be functionally displayed on M13 filamentous phage as a protein III **fusion protein** with retained cellulose binding activity. A combinatorial library comprising 46 million variants of the **CBD** domain was constructed through randomization of 11 positions located at the domain surface and distributed over three separate beta-sheets of the domain. Using the enzyme porcine alpha-amylase (PPA) as target in biopannings, two **CBD** variants showing selective binding to the enzyme were characterized. Reduction and iodoacetamide blocking of cysteine residues in selected **CBD** variants resulted in a loss of binding activity, indicating a conformation dependent binding. Interestingly, further studies showed that the selected **CBD** variants were capable of competing with the binding of the amylase inhibitor acarbose to the enzyme. In addition, the enzyme activity could be partially inhibited by addition of soluble protein, suggesting that the selected **CBD** variants bind to the active site of the enzyme.

L3 ANSWER 35 OF 224 MEDLINE on STN DUPLICATE 53
ACCESSION NUMBER: 2000275530 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10814589
TITLE: Expression, purification and applications of staphylococcal protein A fused to cellulose-binding domain.
AUTHOR: Shpigel E; Goldlust A; Eshel A; Ber I K; Efroni G; Singer Y; Levy I; Dekel M; Shoseyov O
CORPORATE SOURCE: The Kennedy Leigh Centre for Horticulture Research and The Otto Warburg Center for Agricultural Biotechnology, The Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, Israel.
SOURCE: Biotechnology and applied biochemistry, (2000 Jun) Vol. 31 (Pt 3), pp. 197-203.

Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 10 Aug 2000
Last Updated on STN: 10 Aug 2000
Entered Medline: 27 Jul 2000

AB Because staphylococcal Protein A (ProtA) binds specifically to IgG, it has been used for many immunological manipulations, most notably antibody purification and diagnostics. Immobilization is required for most of these applications. Here we describe a genetic-engineering approach to immobilizing ProtA on cellulose, by fusing it to cellulose-binding domain (**CBD**) derived from the cellulose-binding Protein A of Clostridium cellulovorans. The bifunctional **fusion protein** was expressed in Escherichia coli, recovered on a cellulose column and purified by elution at alkaline pH. ProtA-**CBD** was used to purify IgG from rabbit serum and its ability to bind IgG from different sources was determined. The bifunctional chimaeric protein can bind up to 23.4 mg/ml human IgG at a ratio of 1 mol of ProtA-**CBD**/2 mol of human IgG, and can purify up to 11.6 mg/ml rabbit IgG from a serum. The ability to bind functionally active **CBD**-affinity reagents to cellulosic microtitre plates was demonstrated. Our results indicate that a combination of **CBD** -affinity reagents and cellulosic microtitre plates is an attractive diagnostics matrix for the following reasons: (i) cellulose exhibits very low non-specific binding; and (ii) **CBD-fusion proteins** bind directly to cellulose at high density. A unique signal-amplification method was developed based on the ability of ProtA-**CBD** to link stained cellulose particles to primary antibody in a Western blot.

L3 ANSWER 36 OF 224 MEDLINE on STN DUPLICATE 54
ACCESSION NUMBER: 2001073189 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11049737
TITLE: Factor X **fusion proteins**: improved production and use in the release in vitro of biologically active hirudin from an inactive alpha-factor-hirudin **fusion protein**.
AUTHOR: Guarna M M; Cote H C; Kwan E M; Rintoul G L; Meyhack B; Heim J; MacGillivray R T; Warren R A; Kilburn D G
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada.
SOURCE: Protein expression and purification, (2000 Nov) Vol. 20, No. 2, pp. 133-41.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 26 Oct 2002
Entered Medline: 3 Jan 2001

AB Many recombinant proteins are synthesized as **fusion proteins** containing affinity tags to aid in the downstream processing. After purification, the affinity tag is often removed by using a site-specific protease such as factor Xa (FXa). However, the use of FXa is limited by its expense and availability from plasma. To develop a recombinant source of FXa, we have expressed two novel forms of FXa using baby hamster kidney (BHK) cells as host and the expression vector pNUT. The chimeric protein FIIFX consisted of the prepropeptide and the

Gla domain of prothrombin linked to the activation peptide and protease region of FXa, together with a cellulose-binding domain (**CBD** (Cex)) as an affinity tag. A second variant consisted of the transferrin signal peptide linked to the second epidermal growth factor-like domain and the catalytic domain of FX and a polyhistidine tag. Both FX variants were secreted into the medium, their affinity tags were functional, and following activation, both retained FXa-specific proteolytic activity. However, the yield of the FIIFX-**CBD**(Cex) **fusion protein** was 10-fold higher than that of FX-**CBD**(Cex) and other forms of recombinant FX reported to date. The FXa derivatives were used to cleave two different **fusion proteins**, including a biologically inactive alpha-factor-hirudin **fusion protein** secreted by Saccharomyces cerevisiae. After cleavage, the released hirudin demonstrated biological activity in a thrombin inhibition assay, suggesting that this method may be applicable to the production of toxic or unstable proteins. The availability of novel FX derivatives linked to different affinity tags allows the development of a versatile system for processing **fusion proteins** in vitro.

L3 ANSWER 37 OF 224 MEDLINE on STN DUPLICATE 58
ACCESSION NUMBER: 2000014693 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10545273
TITLE: Matrix-assisted refolding of single-chain Fv- cellulose binding domain **fusion proteins**.
AUTHOR: Berdichevsky Y; Lamed R; Frenkel D; Gophna U; Bayer E A; Yaron S; Shoham Y; Benhar I
CORPORATE SOURCE: Department of Molecular Microbiology and Biotechnology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, 69978, Israel.
SOURCE: Protein expression and purification, (1999 Nov) Vol. 17, No. 2, pp. 249-59.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 24 Jan 2000
Last Updated on STN: 24 Jan 2000
Entered Medline: 12 Jan 2000

AB We describe a method for the isolation of recombinant single-chain antibodies in a biologically active form. The single-chain antibodies are fused to a cellulose binding domain as a single-chain protein that accumulates as insoluble inclusion bodies upon expression in Escherichia coli. The inclusion bodies are then solubilized and denatured by an appropriate chaotropic solvent, then reversibly immobilized onto a cellulose matrix via specific interaction of the matrix with the cellulose binding domain (**CBD**) moiety. The efficient immobilization that minimizes the contact between folding protein molecules, thus preventing their aggregation, is facilitated by the robustness of the Clostridium thermocellum **CBD** we use. This **CBD** is unique in retaining its specific cellulose binding capability when solubilized in up to 6 M urea, while the proteins fused to it are fully denatured. Refolding of the **fusion proteins** is induced by reducing with time the concentration of the denaturing solvent while in contact with the cellulose matrix. The refolded single-chain antibodies in their native state are then recovered by releasing them from the cellulose matrix in high yield of 60% or better, which is threefold or higher than the yield obtained by using published refolding protocols to recover the same scFvs. The described method should have general applicability for the production of many protein-**CBD** fusions in which the fusion partner is insoluble upon expression.

L3 ANSWER 38 OF 224 MEDLINE on STN DUPLICATE 59
ACCESSION NUMBER: 1999429353 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10501245
TITLE: Expression of a recombinant form of the V antigen of Yersinia pestis, using three different expression systems.
AUTHOR: Carr S; Miller J; Leary S E; Bennett A M; Ho A; Williamson E D
CORPORATE SOURCE: Defence Evaluation and Research Agency, CBD, Salisbury, Wiltshire, UK.. 100432.3200@compuserve.com
SOURCE: Vaccine, (1999 Aug 20) Vol. 18, No. 1-2, pp. 153-9.
Journal code: 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 11 Jan 2000
Last Updated on STN: 11 Jan 2000
Entered Medline: 28 Oct 1999

AB Yersinia pestis, the causative organism of plague, produces V antigen (LcrV), a bifunctional protein with regulatory and virulence roles that has been shown to be highly protective against a plague challenge. A combined sub-unit vaccine, comprising recombinant V and Fraction 1 antigens is currently being developed. We report here the expression and purification of recombinant V antigen (rV) using three different expression systems: the N-terminal GST fusion pGEX-5X-2 and pGEX-6P-2 systems from Pharmacia Biotech, and the C-terminal **CBD** fusion (IMPACT I) system from New England Biolabs. After cleavage from the carrier protein, the yields of rV were 25 mg l(-1) (pGEX-5X-2), 31 mg l(-1) (pGEX-6P-2) and 0.75 mg l(-1) (IMPACT I). All of the recombinant proteins were immunogenic in mice, although there were some differences in their protective efficacy against subcutaneous challenge with Y. pestis. Whilst rV antigen derived from the IMPACT I and pGEX-6P-2 systems and given in two immunising doses protected fully against challenge with 1 x 10(7) colony forming units (cfu) of Y. pestis, there was breakthrough in protection against 1 x 10(5) cfu of Y. pestis in animals immunised twice with rV from the pGEX-5X-2 system. From this study, the pGEX-6P-2 has been selected for the production of rV as a vaccine component. The pGEX-6P-2 system utilises a GST tagged PreScission Protease (a recombinant human rhinovirus 3C protease) to cleave the **fusion protein**, thereby allowing efficient removal of the enzyme from the final product. In addition, the enzyme is not of animal origin, therefore making it suitable for vaccine production.

L3 ANSWER 39 OF 224 MEDLINE on STN DUPLICATE 60
ACCESSION NUMBER: 2000027007 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10556552
TITLE: Phage display of a cellulose binding domain from Clostridium thermocellum and its application as a tool for antibody engineering.
AUTHOR: Berdichevsky Y; Ben-Zeev E; Lamed R; Benhar I
CORPORATE SOURCE: Department of Molecular Microbiology, The George S. Wise Faculty of Life Sciences, Green Building, Room 202, Tel-Aviv University, Ramat Aviv 69978, Israel.
SOURCE: Journal of immunological methods, (1999 Aug 31) Vol. 228, No. 1-2, pp. 151-62.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 13 Jan 2000
Last Updated on STN: 13 Jan 2000
Entered Medline: 28 Dec 1999

AB Phage display of antibody fragments has proved to be a powerful tool for the isolation and in vitro evolution of these biologically important molecules. However, the general usefulness of this technology is still limited by some technical difficulties. One of the most debilitating obstacles to the widespread application of the technology is the accumulation of "insert loss" clones in the libraries; phagemid clones from which the DNA encoding part or all of the cloned antibody fragment had been deleted. Another difficulty arises when phage technology is applied for cloning hybridoma-derived antibody genes, where myeloma derived light chains, irrelevant to the hybridoma's antibody specificity may be fortuitously cloned. Here, we report the construction of a novel phage-display system designed to address these problems. In our system a single-chain Fv (scFv) is expressed as an in-frame **fusion protein** with a cellulose-binding domain (**CBD**) derived from the Clostridium thermocellum cellulosome. The **CBD** domain serves as an affinity tag allowing rapid phage capture and concentration from crude culture supernatants, and immunological detection of both displaying phage and soluble scFv produced thereof. We demonstrate the utility of our system in solving the technical difficulties described above, and in speeding up the process of scFv isolation from combinatorial antibody repertoires.

L3 ANSWER 40 OF 224 MEDLINE on STN DUPLICATE 61
ACCESSION NUMBER: 1999075936 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9857214
TITLE: Two domains of rat galectin-4 bind to distinct structures of the intercellular borders of colorectal epithelia.
AUTHOR: Wasano K; Hirakawa Y
CORPORATE SOURCE: Department of Anatomy and Cell Biology, Faculty of Medicine, Kyushu University, Fukuoka, Japan.
SOURCE: The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, (1999 Jan) Vol. 47, No. 1, pp. 75-82.
Journal code: 9815334. ISSN: 0022-1554.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 23 Feb 1999
Last Updated on STN: 18 Dec 2002
Entered Medline: 9 Feb 1999

AB Galectin-4 (G4) is a member of a family of soluble galactoside-binding lectins found in various mammalian tissues. To determine the function of this protein in colorectal tissue, we separately produced the N- and C-terminal carbohydrate binding domains (**CBD**) of rat G4 as a recombinant glutathione S-transferase (GST) **fusion protein** (G4-N and G4-C) and examined the tissue binding site(s) of each **CBD** by light and electron microscopy (LM and EM). At the LM level, both **fusion proteins** stained the intercellular borders of the surface-lining epithelial cells of colorectal mucosa. At the EM level, two proteins recognized spatially close but distinct subcellular structures. G4-N stained electron-lucent flocculent substances freely located in the intercellular spaces, whereas G4-C bound to the lateral cell membranes demarcating the intercellular spaces. These findings suggest that colorectal G4 may be involved in crosslinking the lateral cell membranes of the surface-lining epithelial cells, thereby reinforcing epithelial integrity against mechanical stress exerted by the bowel lumen. (J Histochem Cytochem 47:75-82, 1999)

L3 ANSWER 41 OF 224 MEDLINE on STN DUPLICATE 62
ACCESSION NUMBER: 1999369648 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10440667
TITLE: Immobilization of recombinant heparinase I fused to cellulose-binding domain.
AUTHOR: Shpigel E; Goldlust A; Efroni G; Avraham A; Eshel A; Dekel M; Shoseyov O
CORPORATE SOURCE: The Kennedy Leigh Centre for Horticultural Research and The Otto Warburg Center for Agricultural Biotechnology, The Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel.
SOURCE: Biotechnology and bioengineering, (1999 Oct 5) Vol. 65, No. 1, pp. 17-23.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 1 Nov 1999
Last Updated on STN: 1 Nov 1999
Entered Medline: 18 Oct 1999

AB Immobilization of biologically active proteins is of great importance to research and industry. Cellulose is an attractive matrix and cellulose-binding domain (**CBD**) an excellent affinity tag protein for the purification and immobilization of many of these proteins. We constructed two vectors to enable the cloning and expression of proteins fused to the N- or C-terminus of **CBD**. Their usefulness was demonstrated by fusing the heparin-degrading protein heparinase I to **CBD** (**CBD**-HepI and HepI-**CBD**). The **fusion proteins** were over-expressed in Escherichia coli under the control of a T7 promoter and found to accumulate in inclusion bodies. The inclusion bodies were recovered by centrifugation, the proteins were refolded and recovered on a cellulose column. The bifunctional **fusion protein** retained its abilities to bind to cellulose and degrade heparin. C-terminal fusion of heparinase I to **CBD** was somewhat superior to N-terminal fusion: Although specific activities in solution were comparable, the latter exhibited impaired binding capacity to cellulose. **CBD**-HepI-cellulose bioreactor was operated continuously and degraded heparin for over 40 h without any significant loss of activity. By varying the flow rate, the mean molecular weight of the heparin oligosaccharide produced could be controlled. The molecular weight distribution profiles, obtained from heparin depolymerization by free heparinase I, free **CBD**-HepI, and cellulose-immobilized **CBD**-HepI, were compared. The profiles obtained by free heparinase I and **CBD**-HepI were indistinguishable, however, immobilized **CBD**-HepI produced much lower molecular weight fragments at the same percentage of depolymerization. Thus, **CBD** can be used for the efficient production of bioreactors, combining purification and immobilization into essentially a single step.

L3 ANSWER 42 OF 224 MEDLINE on STN DUPLICATE 64
ACCESSION NUMBER: 1998284050 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9618531
TITLE: Collagen-binding growth factors: production and characterization of functional **fusion proteins** having a collagen-binding domain.
AUTHOR: Nishi N; Matsushita O; Yuube K; Miyanaka H; Okabe A; Wada F
CORPORATE SOURCE: Department of Endocrinology, Faculty of Medicine, Kagawa Medical University, Kagawa 761-0793, Japan..

nnishi@kms.ac.jp
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Jun 9) Vol. 95, No. 12, pp. 7018-23.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 16 Jul 1998
Last Updated on STN: 3 Mar 2000
Entered Medline: 9 Jul 1998

AB The autocrine/paracrine peptide signaling molecules such as growth factors have many promising biologic activities for clinical applications. However, one cannot expect specific therapeutic effects of the factors administered by ordinary drug delivery systems as they have limited target specificity and short half-lives in vivo. To overcome the difficulties in using growth factors as therapeutic agents, we have produced **fusion proteins** consisting of growth factor moieties and a collagen-binding domain (**CBD**) derived from Clostridium histolyticum collagenase. The **fusion proteins** carrying the epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) at the N terminal of **CBD** (CBEGF/CBFGF) tightly bound to insoluble collagen and stimulated the growth of BALB/c 3T3 fibroblasts as much as the unfused counterparts. CBEGF, when injected subcutaneously into nude mice, remained at the sites of injection for up to 10 days, whereas EGF was not detectable 24 h after injection. Although CBEGF did not exert a growth-promoting effect in vivo, CBFGF, but not bFGF, strongly stimulated the DNA synthesis in stromal cells at 5 days and 7 days after injection. These results indicate that **CBD** may be used as an anchoring unit to produce **fusion proteins** nondiffusible and long-lasting in vivo.

L3 ANSWER 43 OF 224 MEDLINE on STN DUPLICATE 65
ACCESSION NUMBER: 1999201269 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10099473
TITLE: Improved immobilization of **fusion proteins** via cellulose-binding domains.
AUTHOR: Linder M; Nevanen T; Soderholm L; Bengs O; Teeri T T
CORPORATE SOURCE: VTT Biotechnology and Food Research, P.O. Box 1500, FIN-02044, Finland.
SOURCE: Biotechnology and bioengineering, (1998 Dec 5) Vol. 60, No. 5, pp. 642-7.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 11 May 1999
Last Updated on STN: 11 May 1999
Entered Medline: 26 Apr 1999

AB Cellulose-binding domains (**CBDs**) are structurally and functionally independent, noncatalytic modules found in many cellulose or hemicellulose degrading enzymes. Recent biotechnological applications of the **CBDs** include facilitated protein immobilization on cellulose supports. In some occasions there have been concerns about the stability of the **CBD** driven immobilization. Here we have studied the chromatographic behavior of variants of the Trichoderma reesei cellobiohydrolase I **CBD** belonging to family I. Both **CBDs** fused to antibody fragments and isolated **CBDs** were studied and compared. Tritium labeling by reductive methylation was used

as a sensitive detection method. The **fusion protein** as well as the isolated **CBD** was found to leak from the column at a rate of 0.3-0.5% of the immobilized protein per column volume. However, the leakage could be overcome by using two **CBDs** instead of a single **CBD** for the immobilization. In this way leakage was reduced to less than 0.01% per column volume. The improved immobilization could also be seen as a decreased migration of the protein down the column in extended washes.

L3 ANSWER 44 OF 224 MEDLINE on STN DUPLICATE 66
ACCESSION NUMBER: 2003042183 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12549414
TITLE: Subcloning and expression of coding region for **cellulase binding domain** of CBH I from P. janthinellum in E. coli.
AUTHOR: Wang T; Wang C; Gao P; Zhong L; Zou Y
CORPORATE SOURCE: State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100.
SOURCE: Wei sheng wu xue bao = Acta microbiologica Sinica, (1998 Aug) Vol. 38, No. 4, pp. 269-75.
Journal code: 21610860R. ISSN: 0001-6209.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 29 Jan 2003
Last Updated on STN: 17 Dec 2003
Entered Medline: 21 Nov 2003
AB The in vitro DNA manipulations, included the nested deletions, of cbh1 from P. janthinellum inserted into pUC18-181 were carried out. The two ends of fragments were modified into blunt ends and the fragments were self-ligated. Then, the encircled plasmids were transformed to E. coli JM109. Utilizing the characterization of **CBD** binding to crystalline cellulose, one catalytic domain deletion transformant producing active LacZ-**CBD fusion protein** was isolated from 24 transformants randomly picked from 400 transformants. The molecular weight of the LacZ-**CBD fusion protein** is 21 kD. The plasmid was designated pUC 18C. The LacZ-**CBD fusion protein** produced by JM109(pUC18C) was able to be purified by procedure of adsorption-desorption to cellulose. The pNPC activity of crude enzyme solution of JM109(pUC18C) induced by IPTG were zero, identified the JM109(pUC18C) has no CBHI activity.

L3 ANSWER 45 OF 224 MEDLINE on STN DUPLICATE 67
ACCESSION NUMBER: 1999246634 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10230019
TITLE: Functional analysis of a hybrid endoglucanase of bacterial origin having a cellulose binding domain from a fungal exoglucanase.
AUTHOR: Kim H; Goto M; Jeong H J; Jung K H; Kwon I; Furukawa K
CORPORATE SOURCE: Department of Agricultural Chemistry, Sunchon National University, Korea.
SOURCE: Applied biochemistry and biotechnology, (1998 Nov-Dec) Vol. 75, No. 2-3, pp. 193-204.
Journal code: 8208561. ISSN: 0273-2289.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906

ENTRY DATE: Entered STN: 18 Jun 1999
Last Updated on STN: 3 Mar 2000
Entered Medline: 9 Jun 1999

AB A cellulose binding domain (**CBD**) of an endo-beta-1,4-glucanase (Ben) from the bacterium Bacillus subtilis BSE616 was replaced with the **CBD** of exoglucanase I (TexI) from the fungus Trichoderma viride HK-75. The resultant hybrid enzyme Ben'-CBDTexI, comprising the catalytic domain (Ben') of Ben and the **CBD** (CBDTexI) of TexI, was highly expressed at 20% of the total protein in Escherichia coli. The molecular mass of the hybrid enzyme was estimated to be ca. 38 kDa by SDS-PAGE, which was in good agreement with that calculated from 305 amino acids of Ben and 42 amino acids of CBDTexI. The hybrid enzyme exhibited almost the same activity as that of the original Ben toward soluble substrates, such as cellooligosaccharides. The hybrid enzyme showed higher binding ability and hydrolysis activity toward microcrystalline cellulose (Avicel), even though the length of the **CBD** of TexI was four times smaller than that of Ben. The hybrid enzyme was more resistant to tryptic digestion than the original Ben. The efficient binding ability of the hybrid enzyme to Avicel permitted purification of the enzyme using an Avicel-affinity column to the extent of ca. 90% purity.

L3 ANSWER 46 OF 224 MEDLINE on STN DUPLICATE 69
ACCESSION NUMBER: 97406911 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9260284
TITLE: Xylanase XynA from the hyperthermophilic bacterium Thermotoga maritima: structure and stability of the recombinant enzyme and its isolated cellulose-binding domain.
AUTHOR: Wassenberg D; Schurig H; Liebl W; Jaenicke R
CORPORATE SOURCE: Institut fur Biophysik und Physikalische Biochemie, Universitat Regensburg, Germany.
SOURCE: Protein science : a publication of the Protein Society, (1997 Aug) Vol. 6, No. 8, pp. 1718-26.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 8 Oct 1997
Last Updated on STN: 6 Feb 1998
Entered Medline: 25 Sep 1997

AB The hyperthermophilic bacterium Thermotoga maritima is capable of gaining metabolic energy utilizing xylan. XynA, one of the corresponding hydrolases required for its degradation, is a 120-kDa endo-1,4-D-xylanase exhibiting high intrinsic stability and a temperature optimum approximately 90 degrees C. Sequence alignments with other xylanases suggest the enzyme to consist of five domains. The C-terminal part of XynA was previously shown to be responsible for cellulose binding (Winterhalter C, Heinrich P, Candussio A, Wich G, Liebl W. 1995. Identification of a novel cellulose-binding domain within the multi-domain 120 kDa Xylanase XynA of the hyperthermophilic bacterium Thermotoga maritima. Mol Microbiol 15:431-444). In order to characterize the domain organization and the stability of XynA and its C-terminal cellulose-binding domain (**CBD**), the two separate proteins were expressed in Escherichia coli. **CBD**, because of its instability in its ligand-free form, was expressed as a glutathione S-transferase **fusion protein** with a specific thrombin cleavage site as linker. XynA and **CBD** were compared regarding their hydrodynamic and spectral properties. As taken from analytical ultracentrifugation and gel permeation chromatography, both are monomers with 116 and 22 kDa molecular masses, respectively. In the presence of glucose as a ligand, **CBD** shows high intrinsic stability. Denaturation/renaturation

experiments with isolated **CBD** yield > 80% renaturation, indicating that the domain folds independently. Making use of fluorescence emission and far-UV circular dichroism in order to characterize protein stability, guanidine-induced unfolding of XynA leads to biphasic transitions, with half-concentrations c1/2 (GdmCl) approximately 4 M and > 5 M, in accordance with the extreme thermal stability. At acid pH, XynA exhibits increased stability, indicated by a shift of the second guanidine-transition from 5 to 7 M GdmCl. This can be tentatively attributed to the cellulose-binding domain. Differences in the transition profiles monitored by fluorescence emission and dichroic absorption indicate multi-state behavior of XynA. In the case of **CBD**, a temperature-induced increase in negative ellipticity at 217 nm is caused by alterations in the environment of aromatic residues that contribute to the far-UV CD in the native state.

L3 ANSWER 47 OF 224 MEDLINE on STN DUPLICATE 73
ACCESSION NUMBER: 97368133 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9224900
TITLE: Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element.
AUTHOR: Chong S; Mersha F B; Comb D G; Scott M E; Landry D; Vence L M; Perler F B; Benner J; Kucera R B; Hirvonen C A; Pelletier J J; Paulus H; Xu M Q
CORPORATE SOURCE: New England Biolabs, Beverly, MA 01915, USA.
SOURCE: Gene, (1997 Jun 19) Vol. 192, No. 2, pp. 271-81. Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 25 Aug 1997
Last Updated on STN: 26 Oct 2002
Entered Medline: 8 Aug 1997

AB A novel protein purification system has been developed which enables purification of free recombinant proteins in a single chromatographic step. The system utilizes a modified protein splicing element (intein) from Saccharomyces cerevisiae (Sce VMA intein) in conjunction with a chitin-binding domain (**CBD**) from Bacillus circulans as an affinity tag. The concept is based on the observation that the modified Sce VMA intein can be induced to undergo a self-cleavage reaction at its N-terminal peptide linkage by 1,4-dithiothreitol (DTT), beta-mercaptoethanol (beta-ME) or cysteine at low temperatures and over a broad pH range. A target protein is cloned in-frame with the N-terminus of the intein-**CBD** fusion, and the stable **fusion protein** is purified by adsorption onto a chitin column. The immobilized **fusion protein** is then induced to undergo self-cleavage under mild conditions, resulting in the release of the target protein while the intein-**CBD** fusion remains bound to the column. No exogenous proteolytic cleavage is needed. Furthermore, using this procedure, the purified free target protein can be specifically labeled at its C-terminus.

L3 ANSWER 48 OF 224 MEDLINE on STN DUPLICATE 76
ACCESSION NUMBER: 95234035 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7717975
TITLE: A modular xylanase containing a novel non-catalytic xylan-specific binding domain.
AUTHOR: Black G W; Hazlewood G P; Millward-Sadler S J; Laurie J I; Gilbert H J
CORPORATE SOURCE: Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, U.K.

SOURCE: The Biochemical journal, (1995 Apr 1) Vol. 307 (Pt 1), pp. 191-5.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X76729
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 24 May 1995
Last Updated on STN: 18 Mar 2003
Entered Medline: 18 May 1995

AB Xylanase D (XYLD) from Cellulomonas fimi contains a C-terminal cellulose-binding domain (**CBD**) and an internal domain that exhibits 65% sequence identity with the C-terminal **CBD**. Full-length XYLD binds to both cellulose and xylan. Deletion of the C-terminal **CBD** from XYLD abolishes the capacity of the enzyme to bind to cellulose, although the truncated xylanase retains its xylan-binding properties. A derivative of XYLD lacking both the C-terminal **CBD** and the internal **CBD** homologue did not bind to either cellulose or xylan. A **fusion protein** consisting of the XYLD internal **CBD** homologue linked to the C-terminus of glutathione S-transferase (GST) bound to xylan, but not to cellulose, while GST bound to neither of the polysaccharides. The Km and specific activity of full-length XYLD and truncated derivatives of the enzyme lacking the C-terminal **CBD** (XYLDcbd), and both the **CBD** and the internal **CBD** homologue (XYLDcd), were determined with soluble and insoluble xylan as the substrates. The data showed that the specific activities of the three enzymes were similar for both substrates, as were the Km values for soluble substrate. However, the Km values of XYLD and XYLDcbd for insoluble xylan were significantly lower than the Km of XYLDcd. Overall, these data indicate that the internal **CBD** homologue in XYLD constitutes a discrete xylan-binding domain which influences the affinity of the enzyme for insoluble xylan but does not directly affect the catalytic activity of the xylanase. The rationale for the evolution of this domain is discussed.

L3 ANSWER 49 OF 224 MEDLINE on STN DUPLICATE 78
ACCESSION NUMBER: 95290897 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7772950
TITLE: Purification of human interleukin-2 using the cellulose-binding domain of a prokaryotic cellulase.
AUTHOR: Ong E; Alimonti J B; Greenwood J M; Miller R C Jr; Warren R A; Kilburn D G
CORPORATE SOURCE: Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada.
SOURCE: Bioseparation, (1995 Apr) Vol. 5, No. 2, pp. 95-104.
Journal code: 9011423. ISSN: 0923-179X.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 20 Jul 1995
Last Updated on STN: 20 Jul 1995
Entered Medline: 12 Jul 1995

AB Engineering gene fusions which introduce an affinity tag linked to the target polypeptide by a specific protease cleavage site is widely used to facilitate recombinant protein purification. A **fusion protein** CBDAPT-IL-2, comprised of the cellulose-binding domain (**CBD**) and Pro-Thr (PT) rich linker of the Cellulomonas fimi endo-beta-1,4-glucanase A (CenA) and a factor Xa cleavage sequence (IleGluGlyArg) fused to the N terminus of human interleukin-2, was

produced in Escherichia coli, Streptomyces lividans and mammalian COS cells. CBDAPT-IL-2, secreted from S. lividans or COS cells or recovered from the insoluble fraction of E. coli, could be purified by adsorption on cellulose. The intact **fusion protein** adsorbed to cellulose was hydrolyzed in situ with factor Xa to release active interleukin-2.

L3 ANSWER 50 OF 224 MEDLINE on STN DUPLICATE 79
ACCESSION NUMBER: 95209798 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7765987
TITLE: Processing of **fusion proteins** with immobilized factor Xa.
AUTHOR: Assouline Z; Graham R; Miller R C Jr; Warren A J; Kilburn D G
CORPORATE SOURCE: Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada.
SOURCE: Biotechnology progress, (1995 Jan-Feb) Vol. 11, No. 1, pp. 45-9.
Journal code: 8506292. ISSN: 8756-7938.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 9 Aug 1995
Last Updated on STN: 9 Aug 1995
Entered Medline: 3 May 1995
AB Factor Xa, with a cellulose-binding domain (**CBD**) fused to the C-terminus of the heavy chain (FXa-**CBD**), is active in solution and when immobilized on cellulose. A second derivative of factor Xa in which a hexahistidine tail is fused to the C-terminus of the heavy chain (FXa-H6) also retains activity when immobilized, in this case on Ni(2+)-NTA agarose. The stabilities and activities of of FXa-**CBD** and FXa-H6 immobilized on cellulose and Ni(2+)-NTA agarose, respectively, are similar. Immobilized factor Xa derivatives can be used to remove affinity tags from appropriate **fusion proteins** without contaminating the desired product with factor Xa.

L3 ANSWER 51 OF 224 MEDLINE on STN DUPLICATE 83
ACCESSION NUMBER: 94172316 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8126442
TITLE: Cloning of an endo-(1-->4)-beta-glucanase gene, celA, from the rumen bacterium Clostridium sp. ('C. longisporum') and characterization of its product, CelA, in Escherichia coli.
AUTHOR: Mittendorf V; Thomson J A
CORPORATE SOURCE: Department of Microbiology, University of Cape Town, Rondebosch, South Africa.
SOURCE: Journal of general microbiology, (1993 Dec) Vol. 139, No. 12, pp. 3233-42.
Journal code: 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L02868
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 20 Apr 1994
Last Updated on STN: 20 Apr 1994
Entered Medline: 12 Apr 1994
AB A genomic library of Clostridium sp. ('C. longisporum') ATCC 49440 in the host Escherichia coli was screened for endo-beta-glucanases, and plasmids pCM64 and pCM4 were isolated. The nucleotide sequence of a 3620 bp fragment was found to contain a 1548 bp open reading frame (ORF), termed

celA, which encodes an endo-(1-->4)-beta-glucanase, CelA, assigned to family A4. N-terminal amino acid sequence determination revealed that pCM64 encoded the full-length celA gene, including a signal sequence, while pCM4 carried a 5'-truncated celA gene expressed as an N-terminal **fusion protein**, CelA delta N', without a signal sequence. CelA was secreted into the periplasm in E. coli. In this organism, proteolytic cleavage of CelA at or near a putative linker region resulted in the appearance of two active polypeptides of molecular masses 57 and 47 kDa. The former was the full-length enzyme while the latter consisted of the catalytic domain from which the cellulose-binding domain (**CBD**) had been removed (CelA delta **CBD**). The intracellularly-located CelA delta N' was not subject to proteolytic degradation. The pH and temperature optima of CelA were pH 4.8 and 43 degrees C, respectively. CelA hydrolysed barley beta-glucan, lichenan, carboxymethylcellulose and xylan. It showed preferential activity against the larger cellooligosaccharides (cellohexaose and cellopentaose); cellotetraose was the smallest substrate degraded completely.

L3 ANSWER 52 OF 224 MEDLINE on STN DUPLICATE 86
ACCESSION NUMBER: 93028337 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1409557
TITLE: Cellulose-binding domains: potential for purification of complex proteins.
AUTHOR: Greenwood J M; Ong E; Gilkes N R; Warren R A; Miller R C Jr; Kilburn D G
CORPORATE SOURCE: Department of Microbiology, University of British Columbia, Vancouver, Canada.
SOURCE: Protein engineering, (1992 Jun) Vol. 5, No. 4, pp. 361-5. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 22 Jan 1993
Last Updated on STN: 22 Jan 1993
Entered Medline: 12 Nov 1992

AB The endoglucanase CenA and the exoglucanase Cex from Cellulomonas fimi each contain a discrete cellulose-binding domain (**CBD**), at the amino-terminus or carboxyl-terminus respectively. The gene fragment encoding the **CBD** can be fused to the gene of a protein of interest. Using this approach hybrid proteins can be engineered which bind reversibly to cellulose and exhibit the biological activity of the protein partner. Alkaline phosphatase (PhoA) from Escherichia coli, and a beta-glucosidase (Abg) from an Agrobacterium sp. are dimeric proteins. The fusion polypeptides CenA-PhoA and Abg-CBC(Cex) are sensitive to proteolysis at the junctions between the fusion partners. Proteolysis results in a mixture of homo- and heterodimers; these bind to cellulose if one or both of the monomers carry a **CBD**, e.g. CenA-PhoA/CenA-PhoA and CenA-PhoA/PhoA. **CBD** fusion polypeptides could be used in this way to purify polypeptides which associate with the fusion partner.

L3 ANSWER 53 OF 224 MEDLINE on STN DUPLICATE 87
ACCESSION NUMBER: 92061995 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1953672
TITLE: Characterization of hybrid proteins consisting of the catalytic domains of Clostridium and Ruminococcus endoglucanases, fused to Pseudomonas non-catalytic cellulose-binding domains.
AUTHOR: Poole D M; Durrant A J; Hazlewood G P; Gilbert H J
CORPORATE SOURCE: Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, U.K.

SOURCE: The Biochemical journal, (1991 Nov 1) Vol. 279 (Pt 3), pp. 787-92.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 24 Jan 1992
Last Updated on STN: 24 Jan 1992
Entered Medline: 16 Dec 1991

AB The N-terminal 160 or 267 residues of xylanase A from Pseudomonas fluorescens subsp. cellulosa, containing a non-catalytic cellulose-binding domain (**CBD**), were fused to the N-terminus of the catalytic domain of endoglucanase E (EGE') from Clostridium thermocellum. A further hybrid enzyme was constructed consisting of the 347 N-terminal residues of xylanase C (XYLC) from P. fluorescens subsp. cellulosa, which also constitutes a **CBD**, fused to the N-terminus of endoglucanase A (EGA) from Ruminococcus albus. The three hybrid enzymes bound to insoluble cellulose, and could be eluted such that cellulose-binding capacity and catalytic activity were retained. The catalytic properties of the fusion enzymes were similar to EGE' and EGA respectively. Residues 37-347 and 34-347 of XYLC were fused to the C-terminus of EGE' and the 10 amino acids encoded by the multiple cloning sequence of pMTL22p respectively. The two hybrid proteins did not bind cellulose, although residues 39-139 of XYLC were shown previously to constitute a functional **CBD**. The putative role of the P. fluorescens subsp. cellulosa **CBD** in cellulase action is discussed.

L3 ANSWER 54 OF 224 MEDLINE on STN DUPLICATE 88
ACCESSION NUMBER: 91291333 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1367528
TITLE: Enzyme immobilization using a cellulose-binding domain: properties of a beta-glucosidase **fusion protein**.
AUTHOR: Ong E; Gilkes N R; Miller R C Jr; Warren A J; Kilburn D G
CORPORATE SOURCE: Department of Microbiology, University of British Columbia, Vancouver, Canada.
SOURCE: Enzyme and microbial technology, (1991 Jan) Vol. 13, No. 1, pp. 59-65.
Journal code: 8003761. ISSN: 0141-0229.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 9 Aug 1995
Last Updated on STN: 3 Feb 1997
Entered Medline: 15 Aug 1991

AB Using molecular genetic techniques, a **fusion protein** has been produced which contains the cellulose-binding domain (**CBD**) of an exoglucanase (Cex) from Cellulomonas fimi fused to a beta-glucosidase (Abg) from Agrobacterium sp. The **CBD** functions as an affinity tag for the simultaneous purification and immobilization of the enzyme on cellulose. Binding to cellulose was stable for prolonged periods at temperatures from 4 degrees C to at least 50 degrees C, at ionic strengths from 10 mM to greater than 1 M, and at pH values below 8. The **fusion protein** can be desorbed from cellulose with distilled water or at pH greater than 8. Immobilized enzyme columns of the **fusion protein** bound to cotton fibers exhibited stable beta-glucosidase activity for at least 10 days of continuous operation at temperatures up to 37 degrees C. At higher temperatures, the bound enzyme lost activity. The thermal stability of the **fusion**

protein was greatly improved by immobilization. Immobilization did not alter the pH stability. Except for its ability to bind to cellulose, the properties of the **fusion protein** were virtually the same as those of the native enzyme.

L3 ANSWER 55 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2006020219 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16267041
TITLE: The role of the epsilon subunit in the Escherichia coli ATP synthase. The C-terminal domain is required for efficient energy coupling.
AUTHOR: Cipriano Daniel J; Dunn Stanley D
CORPORATE SOURCE: Department of Biochemistry, University of Western Ontario, London, Ontario N6A 5C1, Canada.
SOURCE: The Journal of biological chemistry, (2006 Jan 6) Vol. 281, No. 1, pp. 501-7. Electronic Publication: 2005-11-02. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200602
ENTRY DATE: Entered STN: 13 Jan 2006
Last Updated on STN: 1 Mar 2006
Entered Medline: 28 Feb 2006

AB The role of the C-domain of the epsilon subunit of ATP synthase was investigated by fusing either the 20-kDa flavodoxin (Fd) or the 5-kDa chitin binding domain (**CBD**) to the N termini of both full-length epsilon and a truncation mutant epsilon(88-stop). All mutant epsilon proteins were stable in cells and supported F1F0 assembly. Cells expressing the Fd-epsilon or Fd-epsilon(88-stop) mutants were unable to grow on acetate minimal medium, indicating their inability to carry out oxidative phosphorylation because of steric blockage of rotation. The other forms of epsilon supported growth on acetate. Membrane vesicles containing Fd-epsilon showed 23% of the wild type ATPase activity but no proton pumping, suggesting that the ATP synthase is intrinsically partially uncoupled. Vesicles containing **CBD**-epsilon were indistinguishable from the wild type in ATPase activity and proton pumping, indicating that the N-terminal fusions alone do not promote uncoupling. Fd-epsilon(88-stop) caused higher rates of uncoupled ATP hydrolysis than Fd-epsilon, and epsilon(88-stop) showed an increased rate of membrane-bound ATP hydrolysis but decreased proton pumping relative to the wild type. Both results demonstrate the role of the C-domain in coupling. Analysis of the wild type and epsilon(88-stop) mutant membrane ATPase activities at concentrations of ATP from 50 mum to 8 mm showed no significant dependence of the ratio of bound/released ATPase activity on ATP concentration. These results support the hypothesis that the main function of the C-domain in the Escherichia coli epsilon subunit is to reduce uncoupled ATPase activity, rather than to regulate coupled activity.

L3 ANSWER 56 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2005524498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16199585
TITLE: Site-directed mutagenesis and expression of the soluble form of the family IIIa cellulose binding domain from the cellulosomal scaffolding protein of Clostridium cellulovorans.
AUTHOR: Murashima Koichiro; Kosugi Akihiko; Doi Roy H
CORPORATE SOURCE: Section of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA.
SOURCE: Journal of bacteriology, (2005 Oct) Vol. 187, No. 20, pp. 7146-9.

Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200511
ENTRY DATE: Entered STN: 4 Oct 2005
Last Updated on STN: 16 Nov 2005
Entered Medline: 15 Nov 2005

AB The planar and anchoring residues of the family IIIa cellulose binding domain (**CBD**) from the cellulosomal scaffolding protein of Clostridium cellulovorans were investigated by site-directed mutagenesis and cellulose binding studies. By fusion with maltose binding protein, the family IIIa recombinant wild-type and mutant **CBDs** from C. cellulovorans were expressed as soluble forms. Cellulose binding tests of the mutant **CBDs** indicated that the planar strip residues played a major role in cellulose binding and that the anchoring residues played only a minor role.

L3 ANSWER 57 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2005643779 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16328992
TITLE: Expression of an insect excitatory toxin, BmK IT, from the scorpion, Buthus martensii Karsch, and its biological activity.
AUTHOR: Hao Chan-juan; Xu Cheng-gang; Wang Wei; Chai Bao-feng; Liang Ai-hua
CORPORATE SOURCE: Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Institute of Biotechnology, Shanxi University, 030006 Taiyuan, P. R. China.
SOURCE: Biotechnology letters, (2005 Dec) Vol. 27, No. 23-24, pp. 1929-34.
Journal code: 8008051. ISSN: 0141-5492.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200604
ENTRY DATE: Entered STN: 6 Dec 2005
Last Updated on STN: 11 Apr 2006
Entered Medline: 10 Apr 2006

AB An insect excitatory toxin from Buthus martensii Karsch (BmK IT) was cloned into the expression vector, pTWIN1, and expressed into Escherichia coli BL21 (DE3) host cells. The soluble fusion expression of **CBD** -intein-BmK IT was obtained. The recombinant BmK IT was purified by two anion-exchange chromatography columns and one gel chromatography column. Bioassays were carried out to verify the toxicity of this recombinant toxin. At the end of a 96 h experimental period, 83% of cotton bollworm larvae were killed with an LT(50) value of 58-62 h. Furthermore, the average weight of larvae fed on BmK IT-containing media was approx 4% of that of the control groups. The results indicate that the expressed and purified recombinant BmK IT has biological activity.

L3 ANSWER 58 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2004039081 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14738848
TITLE: Engineering a bifunctional starch-cellulose cross-bridge protein.
AUTHOR: Levy Ilan; Paldi Tzur; Shoseyov Oded
CORPORATE SOURCE: Faculty of Agricultural, Food and Environmental Quality Sciences, Institute of Plant Science and Genetics in Agriculture, Hebrew University of Jerusalem, P.O. Box 12,

76100, Rehovot, Israel.
SOURCE: Biomaterials, (2004 May) Vol. 25, No. 10, pp. 1841-9.
Journal code: 8100316. ISSN: 0142-9612.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200409
ENTRY DATE: Entered STN: 24 Jan 2004
Last Updated on STN: 15 Sep 2004
Entered Medline: 14 Sep 2004

AB Biodegradable starch- and cellulose-based polymers have a range of properties which make them suitable for use in a wide array of biomedical applications ranging from bone replacement to engineering of tissue scaffolds and drug delivery systems. A novel polysaccharide cross-bridging protein was designed which was comprised of a cellulose-binding domain from Clostridium cellulovorans (**CBD** (clos)) and a starch-binding domain from Aspergillus niger B1 (SBD(Asp)). The two genes were fused in-frame via a synthetic elastin gene to construct a Cellulose/Starch Cross bridging Protein (CSCP). Recombinant CSCP was expressed in Escherichia coli, and successfully refolded from inclusion bodies. CSCP demonstrated cross-bridging ability in different model systems composed of insoluble or soluble starch and cellulose. The aspect that different carbohydrate-binding module maintain their binding capacity over a wide range of conditions, without the need for chemical reactions, makes them attractive domains for designing new classes of chimeric polysaccharide-binding domains which demonstrate potential for use in a wide range of biomaterials.

L3 ANSWER 59 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2004009298 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14706656
TITLE: The carbohydrate-binding domain of Lafora disease protein targets Lafora polyglucosan bodies.
AUTHOR: Ganesh Subramaniam; Tsurutani Naomi; Suzuki Toshimitsu; Hoshii Yoshinobu; Ishihara Tokuhiro; Delgado-Escueta Antonio V; Yamakawa Kazuhiro
CORPORATE SOURCE: Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India.. sganesh@iitk.ac.in
SOURCE: Biochemical and biophysical research communications, (2004 Jan 23) Vol. 313, No. 4, pp. 1101-9.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY452479
ENTRY MONTH: 200403
ENTRY DATE: Entered STN: 7 Jan 2004
Last Updated on STN: 6 Mar 2004
Entered Medline: 5 Mar 2004

AB Lafora's disease (LD) is an autosomal recessive and fatal form of epilepsy with onset in late childhood or adolescence. One of the characteristic features of LD pathology is the presence of periodic acid-Schiff (PAS) positive Lafora inclusion bodies. Lafora bodies are present primarily in neurons, but they have also been found in other organs. Histochemical and biochemical studies have indicated that Lafora bodies are composed mainly of polysaccharides. The LD gene, EPM2A, encodes a 331 amino acid long protein named laforin that contains an N-terminal carbohydrate-binding domain (**CBD**) and a C-terminal dual-specificity phosphatase domain (DSPD). Here we demonstrate that the **CBD** of laforin

targets the protein to Lafora inclusion bodies and this property could be evolutionarily conserved. We also tested in vitro the effects of five LD missense mutations on laforin's affinity to Lafora body. While the missense mutant W32G failed to bind to purified Lafora body, four other mutants (S25P, E28L, F88L, and R108C) did not show any effect on the binding affinity. Based on these observations we propose the existence of a laforin-mediated glycogen metabolic pathway regulating the disposal of pathogenic polyglucosan inclusions. This is the first report demonstrating a direct association between the LD gene product and the disease-defining storage product, the Lafora bodies.

L3 ANSWER 60 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2004509723 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15221226
TITLE: Production of autoproteolytically subunit-assembled 7-beta-(4-carboxybutanamido)cephalosporanic acid (GL-7ACA) acylase from Pseudomonas sp. C427 using a chitin-binding domain.
AUTHOR: Nagao Koji; Yamashita Michio; Ueda Mitsuyoshi
CORPORATE SOURCE: Fermentation Development Laboratories, Fujisawa Pharmaceutical Co. Ltd, 156, Nakagawara, Shinkawa-cho, 452-0915, Nishikasugai-gun, Aichi, Japan.. koji_nagao@po.fujisawa.co.jp
SOURCE: Applied microbiology and biotechnology, (2004 Sep) Vol. 65, No. 4, pp. 407-13. Electronic Publication: 2004-06-18. Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200502
ENTRY DATE: Entered STN: 14 Oct 2004
Last Updated on STN: 1 Mar 2005
Entered Medline: 25 Feb 2005

AB 7-Beta-(4-Carboxybutanamido)cephalosporanic acid (GL-7ACA) acylase from Pseudomonas sp. C427 is known as a proteolytically processed bacterial enzyme. GL-7ACA acylase from Pseudomonas sp. C427 (C427) consists of alpha- and beta-subunits that are processed from a precursor peptide by removing the spacer peptide. A chitin-binding domain (**CBD**) of chitinase A1 derived from Bacillus circulans was genetically fused into four different positions of the C427-encoding gene. In the four enzymes thereby produced, Nalpha427, SP427, Calpha427, and Cbeta427, it was fused, respectively, to the N-terminal region of the alpha-subunit; the C-terminal region of the alpha-subunit; the three-amino-acid upper region of the C-terminal of the alpha-subunit; and to the C-terminal region of the beta-subunit. All of the fusion enzymes, expressed in Escherichia coli, were successfully processed into active forms and had GL-7ACA acylase activity. The affinity-binding activity to crystalline chitin was affected by the fusing position of **CBD**. Nalpha427, Calpha427, and Cbeta427 remained fused to the **CBD** after their processing steps and could bind to chitin, but in the case of SP427 the fused **CBD** was cleaved away during the processing steps and binding activity was no longer observed. These results indicate that **CBD** is functional in such autoproteolytically subunit-assembled acylases.

L3 ANSWER 61 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2004156725 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15051546
TITLE: Tight attachment of chitin-binding-domain-tagged proteins to surfaces coated with acetylated chitosan.
AUTHOR: Bernard Michael P; Cao Donghui; Myers Rebecca V; Moyle William R
CORPORATE SOURCE: Department of Obstetrics and Gynecology, UMDNJ-Robert Wood

Johnson (Rutgers) Medical School, Piscataway, NJ 08854, USA.
CONTRACT NUMBER: HD14907 (NICHD)
HD38547 (NICHD)
SOURCE: Analytical biochemistry, (2004 Apr 15) Vol. 327, No. 2, pp. 278-83.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200411
ENTRY DATE: Entered STN: 31 Mar 2004
Last Updated on STN: 19 Dec 2004
Entered Medline: 29 Nov 2004

AB Several excellent procedures for trapping tagged proteins have been devised, but many of these are expensive, cannot be used outside a limited pH range, fail to work in the presence of chaotropic agents, or are difficult to use. The chitin binding domain (**CBD**) of Bacillus circulans chitinase, which binds to chitin matrices prepared from inexpensive reagents isolated from crab shells, is an alternative tag that can be used under a variety of pH and denaturing conditions. Kits based on the interaction between the **CBD** and the chitin beads are available commercially. Here, we show that simultaneous treatment of microtiter plates with chitosan, a deacetylated form of chitin, and acetic anhydride produces a surface-bound film of chitin that also interacts tightly with the **CBD**. Chitin-coated microtiter well plates captured a **CBD**-tagged heterodimeric human glycoprotein hormone analog directly from mammalian cell culture media, even when present in trace amounts. Binding to the surface was stable in sodium dodecylsulfate and reversed only partially at low pH or in 8M urea at 37 degrees C. This technique appears well suited to surface attachment and permits biochemical or other analyses of molecules that can be tagged with a **CBD**.

L3 ANSWER 62 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2003451584 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12915448
TITLE: The Lafora disease gene product laforin interacts with HIRIP5, a phylogenetically conserved protein containing a NifU-like domain.
AUTHOR: Ganesh Subramaniam; Tsurutani Naomi; Suzuki Toshimitsu; Ueda Kazunori; Agarwala Kishan Lal; Osada Hiroyuki; Delgado-Escueta Antonio V; Yamakawa Kazuhiro
CORPORATE SOURCE: Laboratory for Neurogenetics, RIKEN Brain Science Institute, 2-1, Hirosawa, Wakoshi 351-0198, Japan.. sganesh@iitk.ac.in
CONTRACT NUMBER: NS42376 (NINDS)
SOURCE: Human molecular genetics, (2003 Sep 15) Vol. 12, No. 18, pp. 2359-68. Electronic Publication: 2003-07-29.
Journal code: 9208958. ISSN: 0964-6906.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200404
ENTRY DATE: Entered STN: 30 Sep 2003
Last Updated on STN: 16 Apr 2004
Entered Medline: 15 Apr 2004

AB Lafora disease is an autosomal recessive type of progressive myoclonus epilepsy caused by mutations in the EPM2A gene. The EPM2A gene-encoded protein laforin is a dual-specificity phosphatase that associates with

polyribosomes. Because the cellular functions of laforin are largely unknown, we used the yeast-two hybrid system to screen for protein(s) that interact with laforin. We found that laforin interacts with a phylogenetically conserved protein HIRIP5 that harbors a NifU-like domain. Both in vitro and in vivo assay have shown that the interaction is specific and that laforin probably uses its N-terminal **CBD**-4 domain to interact with the C-terminal NifU-like domain of the HIRIP5 protein. HIRIP5 encodes a cytosolic protein and is expressed ubiquitously, perhaps reflecting a house-keeping function. The presence of a NifU-like domain in the HIRIP5 protein raises an interesting possibility that it may be involved in iron homeostasis. Although the significance of the interaction between HIRIP5 and laforin proteins is not yet fully known, because laforin dephosphorylated HIRIP5 in vitro, HIRIP5 promises to be an interesting laforin-binding partner and would contribute to the understanding of the molecular pathology of Lafora disease.

L3 ANSWER 63 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2002209477 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11943154
TITLE: Membrane distal cytokine binding domain of LIFR interacts with soluble CNTFR in vitro.
AUTHOR: He Wei; Gong Ke; Zhu Guang; Smith David K; Ip Nancy Y
CORPORATE SOURCE: Department of Biochemistry and Biotechnology Research Institute, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, PR China.
SOURCE: FEBS letters, (2002 Mar 13) Vol. 514, No. 2-3, pp. 214-8. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 12 Apr 2002
Last Updated on STN: 7 May 2002
Entered Medline: 6 May 2002

AB Ciliary neurotrophic factor (CNTF) is a member of the gp130 family of cytokines. The functional receptor complex of CNTF is composed of the CNTF receptor alpha (CNTFR), gp130 and the leukemia inhibitory factor receptor (LIFR). Three regions on CNTF have been identified as binding sites for its receptors. The ligand-receptor interactions are mediated through the cytokine binding domains (**CBDs**) and/or the immunoglobulin-like domains of the receptors. However, in the case of CNTF, the precise nature of the protein-protein contacts in the signaling complex has not yet been resolved. In this study, we provide the first demonstration that the membrane distal **CBD** (CBD1) of LIFR associates in vitro with soluble CNTFR in the absence of CNTF. Moreover, purified CBD1 partially blocks CNTF signaling, but not that of interleukin-6 or LIF, in human embryonal carcinoma cell line Ntera/D1 cells. These data raise the possibility that LIFR has the capability to form a ligand-free complex with CNTFR.

L3 ANSWER 64 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2001524012 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11571172
TITLE: Generation of metal-binding staphylococci through surface display of combinatorially engineered cellulose-binding domains.
AUTHOR: Wernerus H; Lehtio J; Teeri T; Nygren P A; Stahl S
CORPORATE SOURCE: Department of Biotechnology, SCFAB, Kungliga Tekniska Hogskolan, SE-10691 Stockholm, Sweden.
SOURCE: Applied and environmental microbiology, (2001 Oct) Vol. 67, No. 10, pp. 4678-84. Journal code: 7605801. ISSN: 0099-2240.

PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 26 Sep 2001
Last Updated on STN: 22 Jan 2002
Entered Medline: 4 Dec 2001

AB Ni(2+)-binding staphylococci were generated through surface display of combinatorially engineered variants of a fungal cellulose-binding domain (**CBD**) from Trichoderma reesei cellulase Cel7A. Novel **CBD** variants were generated by combinatorial protein engineering through the randomization of 11 amino acid positions, and eight potentially Ni(2+)-binding **CBDs** were selected by phage display technology. These new variants were subsequently genetically introduced into chimeric surface proteins for surface display on Staphylococcus carnosus cells. The expressed chimeric proteins were shown to be properly targeted to the cell wall of S. carnosus cells, since full-length proteins could be extracted and affinity purified. Surface accessibility for the chimeric proteins was demonstrated, and furthermore, the engineered **CBDs**, now devoid of cellulose-binding capacity, were shown to be functional with regard to metal binding, since the recombinant staphylococci had gained Ni(2+)-binding capacity. Potential environmental applications for such tailor-made metal-binding bacteria as bioadsorbents in biofilters or biosensors are discussed.

L3 ANSWER 65 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2001438219 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11483010
TITLE: Cloning, expression, and purification of the Staphylococcus simulans lysostaphin using the intein-chitin-binding domain (**CBD**) system.
AUTHOR: Szweda P; Pladzyk R; Kotlowski R; Kur J
CORPORATE SOURCE: Department of Microbiology, Technical University of Gdansk, ul. Narutowicza 11/12, 80-952 Gdansk, Poland.
SOURCE: Protein expression and purification, (2001 Aug) Vol. 22, No. 3, pp. 467-71.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 29 Oct 2001
Last Updated on STN: 29 Oct 2001
Entered Medline: 25 Oct 2001

AB The Staphylococcus simulans gene encoding lysostaphin has been PCR amplified from pRG5 recombinant plasmid (ATCC 67076) and cloned into Escherichia coli expression pTYB12 vector (IMPACT-CN System, New England BioLabs) which allows the overexpression of a target protein as a fusion to a self-cleavable affinity tag. The self-cleavage activity of the intein allows the release of the lysostaphin enzyme from the chitin-bound intein tag, resulting in a single-column purification of the target protein. This abundant overproduction allows purifying milligram amounts of the enzyme.

L3 ANSWER 66 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2001339873 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11281712
TITLE: Expression in Pichia pastoris of Candida antarctica lipase B and lipase B fused to a cellulose-binding domain.

AUTHOR: Rotticci-Mulder J C; Gustavsson M; Holmquist M; Hult K; Martinelle M
CORPORATE SOURCE: Department of Biotechnology, Royal Institute of Technology, SE-100 44 Stockholm, Sweden.
SOURCE: Protein expression and purification, (2001 Apr) Vol. 21, No. 3, pp. 386-92.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 18 Jun 2001
Last Updated on STN: 18 Jun 2001
Entered Medline: 14 Jun 2001

AB Candida antarctica lipase B (CALB) and C. antarctica lipase B fused to a cellulose-binding domain (**CBD**-CALB) were expressed functionally in the methylotrophic yeast Pichia pastoris. The cellulose-binding domain originates from cellulase A of the anaerobic rumen fungus Neocallimastix patriciarum. The genes were fused to the alpha-factor secretion signal sequence of Saccharomyces cerevisiae and placed under the control of the alcohol oxidase gene (AOX1) promoter. The recombinant proteins were secreted into the culture medium reaching levels of approximately 25 mg/L. The proteins were purified using hydrophobic interaction chromatography and gel filtration with an overall yield of 69%. Results from endoglycosidase H digestion of the proteins showed that CALB and **CBD**-CALB were N-glycosylated. The specific hydrolytic activities of recombinant CALB and **CBD**-CALB were identical to that reported for CALB isolated from its native source. The fusion of the **CBD** to the lipase resulted in a greatly enhanced binding toward cellulose for **CBD**-CALB compared with that for CALB.

L3 ANSWER 67 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2001338161 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11179652
TITLE: Directed immobilization of recombinant staphylococci on cotton fibers by functional display of a fungal cellulose-binding domain.
AUTHOR: Lehtio J; Wernerus H; Samuelson P; Teeri T T; Stahl S
CORPORATE SOURCE: Department of Biotechnology, Royal Institute of Technology (KTH), SE-100 44 Stockholm, Sweden.
SOURCE: FEMS microbiology letters, (2001 Feb 20) Vol. 195, No. 2, pp. 197-204.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 18 Jun 2001
Last Updated on STN: 18 Jun 2001
Entered Medline: 14 Jun 2001

AB The immobilization of recombinant staphylococci onto cellulose fibers through surface display of a fungal cellulose-binding domain (**CBD**) was investigated. Chimeric proteins containing the **CBD** from Trichoderma reesei cellulase Cel6A were found to be correctly targeted to the cell wall of Staphylococcus carnosus cells, since full-length proteins could be extracted and affinity-purified. Furthermore, surface accessibility of the **CBD** was verified using a monoclonal antibody and functionality in terms of cellulose-binding was demonstrated in two different assays in which recombinant staphylococci were found to efficiently bind to cotton fibers. The implications of this strategy of

directed immobilization for the generation of whole-cell microbial tools for different applications will be discussed.

L3 ANSWER 68 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2000179805 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10714996
TITLE: CelE, a multidomain cellulase from Clostridium cellulolyticum: a key enzyme in the cellulosome?.
AUTHOR: Gaudin C; Belaich A; Champ S; Belaich J P
CORPORATE SOURCE: Laboratoire de Bioenergetique et Ingenierie des Proteines, IBSM, Centre National de la Recherche Scientifique, Marseille, France.. gaudin@ibsm.cnrs-mrs.fr
SOURCE: Journal of bacteriology, (2000 Apr) Vol. 182, No. 7, pp. 1910-5.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M87018
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 21 Apr 2000
Last Updated on STN: 21 Apr 2000
Entered Medline: 11 Apr 2000

AB CelE, one of the three major proteins of the cellulosome of Clostridium cellulolyticum, was characterized. The amino acid sequence of the protein deduced from celE DNA sequence led us to the supposition that CelE is a three-domain protein. Recombinant CelE and a truncated form deleted of the putative cellulose binding domain (**CBD**) were obtained. Deletion of the **CBD** induces a total loss of activity. Exhibiting rather low levels of activity on soluble, amorphous, and crystalline celluloses, CelE is more active on p-nitrophenyl-cellobiose than the other cellulases from this organism characterized to date. The main product of its action on Avicel is cellobiose (more than 90% of the soluble sugars released), and its attack on carboxymethyl cellulose is accompanied by a relatively small decrease in viscosity. All of these features suggest that CelE is a cellobiohydrolase which has retained a certain capacity for random attack mode. We measured saccharification of Avicel and bacterial microcrystalline cellulose by associations of CelE with four other cellulases from C. cellulolyticum and found that CelE acts synergistically with all tested enzymes. The positive influence of CelE activity on the activities of other cellulosomal enzymes may explain its relative abundance in the cellulosome.

L3 ANSWER 69 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2000138161 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10671457
TITLE: Feruloyl esterase activity of the Clostridium thermocellum cellulosome can be attributed to previously unknown domains of XynY and XynZ.
AUTHOR: Blum D L; Kataeva I A; Li X L; Ljungdahl L G
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology and the Center for Biological Resource Recovery, The University of Georgia, Athens, Georgia 30602, USA.
SOURCE: Journal of bacteriology, (2000 Mar) Vol. 182, No. 5, pp. 1346-51.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 14 Mar 2000

Last Updated on STN: 14 Mar 2000
Entered Medline: 2 Mar 2000

AB The cellulosome of Clostridium thermocellum is a multiprotein complex with endo- and exocellulase, xylanase, beta-glucanase, and acetyl xylan esterase activities. XynY and XynZ, components of the cellulosome, are composed of several domains including xylanase domains and domains of unknown function (UDs). Database searches revealed that the C- and N-terminal UDs of XynY and XynZ, respectively, have sequence homology with the sequence of a feruloyl esterase of strain PC-2 of the anaerobic fungus Orpinomyces. Purified cellulosomes from C. thermocellum were found to hydrolyze FAXX (O-(5-O-[(E)-feruloyl]-alpha-L-arabinofuranosyl)-(1-->3)-O-beta-D- xyl opyranosyl-(1-->4)-D-xylopyranose) and FAX(3) (5-O-[(E)-feruloyl]-[O-beta-D-xylopyranosyl-(1-->2)]-O-alpha-L-arabinofuranosyl-[1-->3])-O-beta-D-xylopyranosyl-(1-->4)-D-xylopyranose) , yielding ferulic acid as a product, indicating that they have feruloyl esterase activity. Nucleotide sequences corresponding to the UDs of XynY and XynZ were cloned into Escherichia coli, and the expressed proteins hydrolyzed FAXX and FAX(3). The recombinant feruloyl esterase domain of XynZ alone (FAE(XynZ)) and with the adjacent cellulose binding domain (FAE-**CBD**(XynZ)) were characterized. FAE-**CBD**(XynZ) had a molecular mass of 45 kDa that corresponded to the expected product of the 1,203-bp gene. K(m) and V(max) values for FAX(3) were 5 mM and 12.5 U/mg, respectively, at pH 6.0 and 60 degrees C. PAX(3), a substrate similar to FAX(3) but with a p-coumaroyl group instead of a feruloyl moiety was hydrolyzed at a rate 10 times slower. The recombinant enzyme was active between pH 3 to 10 with an optimum between pH 4 to 7 and at temperatures up to 70 degrees C. Treatment of Coastal Bermuda grass with the enzyme released mainly ferulic acid and a lower amount of p-coumaric acid. FAE(XynZ) had similar properties. Removal of the 40 C-terminal amino acids, residues 247 to 286, of FAE(XynZ) resulted in protein without activity. Feruloyl esterases are believed to aid in a release of lignin from hemicellulose and may be involved in lignin solubilization. The presence of feruloyl esterase in the C. thermocellum cellulosome together with its other hydrolytic activities demonstrates a powerful enzymatic potential of this organelle in plant cell wall decomposition.

L3 ANSWER 70 OF 224 MEDLINE on STN
ACCESSION NUMBER: 2000388418 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10844677
TITLE: The thermostabilizing domain of the modular xylanase XynA of Thermotoga maritima represents a novel type of binding domain with affinity for soluble xylan and mixed-linkage beta-1,3/beta-1, 4-glucan.
AUTHOR: Meissner K; Wassenberg D; Liebl W
CORPORATE SOURCE: Institut fur Mikrobiologie und Genetik, Georg-August-Universitat, Gottingen, Germany.
SOURCE: Molecular microbiology, (2000 May) Vol. 36, No. 4, pp. 898-912.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 18 Aug 2000
Last Updated on STN: 18 Aug 2000
Entered Medline: 10 Aug 2000

AB Thermotoga maritima XynA is an extremely thermostable modular enzyme with five domains (A1-A2-B-C1-C2). Its catalytic domain (-B-) is flanked by duplicated non-catalytic domains. The C-terminal repeated domains represent cellulose-binding domains (**CBDs**). Xylanase domains related to the N-terminal domains of XynA (A1-A2) are called thermostabilizing domains because their deletion normally leads to

increased thermosensitivity of the enzymes. It was found that a glutathione-S-transferase (GST) hybrid protein (GST-A1A2) containing both A-domains of XynA can interact with various soluble xylan preparations and with mixed-linkage beta-1,3/beta-1,4-glucans. GST-A1A2 showed no affinity for insoluble microcrystalline cellulose, whereas, vice versa, GST-C2, which contains the C-terminal **CBD** of XynA, did not interact with soluble xylan. Another hybrid protein, GST-A2, displayed the same binding properties as GST-A1A2, indicating that A2 alone can also promote xylan binding. The dissociation constants for the binding of xylose, xylobiose, xylotriose, xylotetraose and xylopentaose by GST-A2, as determined at 20 degrees C by fluorescence quench experiments, were 8.1 x 10(-3) M, 2.3 x 10(-4) M, 2.3 x 10(-5) M, 2.5 x 10(-6)M and 1.1 x 10(-6) M respectively. The A-domains of XynA, which are designated as xylan binding domains (XBD), are, from the structural as well as the functional point of view, prototypes of a novel class of binding domains. More than 50 related protein segments with hitherto unknown function were detected in about 30 other multidomain beta-glycanases, among them putative plant (Arabidopsis thaliana) xylanases. It is argued that polysaccharide binding and not thermostabilization is the main function of A-like domains.

L3 ANSWER 71 OF 224 MEDLINE on STN
ACCESSION NUMBER: 1999386680 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10455036
TITLE: The type II and X cellulose-binding domains of Pseudomonas xylanase A potentiate catalytic activity against complex substrates by a common mechanism.
AUTHOR: Gill J; Rixon J E; Bolam D N; McQueen-Mason S; Simpson P J; Williamson M P; Hazlewood G P; Gilbert H J
CORPORATE SOURCE: Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.
SOURCE: The Biochemical journal, (1999 Sep 1) Vol. 342 (Pt 2), pp. 473-80.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 11 Jan 2000
Last Updated on STN: 17 Sep 2002
Entered Medline: 1 Nov 1999

AB Xylanase A (Pf Xyn10A), in common with several other Pseudomonas fluorescens subsp. cellulosa polysaccharidases, consists of a Type II cellulose-binding domain (**CBD**), a catalytic domain (Pf Xyn10A(CD)) and an internal domain that exhibits homology to Type X **CBDs**. The Type X **CBD** of Pf Xyn10A, expressed as a discrete entity (**CBD**(X)) or fused to the catalytic domain (Pf Xyn10A'), bound to amorphous and bacterial microcrystalline cellulose with a K(a) of 2.5 x 10(5) M(-1). **CBD**(X) exhibited no affinity for soluble forms of cellulose or cello-oligosaccharides, suggesting that the domain interacts with multiple cellulose chains in the insoluble forms of the polysaccharide. Pf Xyn10A' was 2-3 times more active against cellulose-hemicellulose complexes than Pf Xyn10A(CD); however, Pf Xyn10A' and Pf Xyn10A(CD) exhibited the same activity against soluble substrates. **CBD**(X) did not disrupt the structure of plant-cell-wall material or bacterial microcrystalline cellulose, and did not potentiate Pf Xyn10A(CD) when not covalently linked to the enzyme. There was no substantial difference in the affinity of full-length Pf Xyn10A and the enzyme's Type II **CBD** for cellulose. The activity of Pf Xyn10A against cellulose-hemicellulose complexes was similar to that of Pf Xyn10A', and a derivative of Pf Xyn10A in which the Type II **CBD** is linked to the Pf Xyn10A(CD) via a serine-rich linker sequence [Bolam,

Cireula, McQueen-Mason, Simpson, Williamson, Rixon, Boraston, Hazlewood and Gilbert (1998) Biochem J. 331, 775-781]. These data indicate that **CBD**(X) is functional in Pf Xyn10A and that no synergy, either in ligand binding or in the potentiation of catalysis, is evident between the Type II and X **CBDs** of the xylanase.

L3 ANSWER 72 OF 224 MEDLINE on STN
ACCESSION NUMBER: 1998228214 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9560304
TITLE: Pseudomonas cellulose-binding domains mediate their effects by increasing enzyme substrate proximity.
AUTHOR: Bolam D N; Ciruela A; McQueen-Mason S; Simpson P; Williamson M P; Rixon J E; Boraston A; Hazlewood G P; Gilbert H J
CORPORATE SOURCE: Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK.
SOURCE: The Biochemical journal, (1998 May 1) Vol. 331 (Pt 3), pp. 775-81.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 6 Jan 1999
Last Updated on STN: 17 Sep 2002
Entered Medline: 26 Oct 1998

AB To investigate the mode of action of cellulose-binding domains (**CBDs**), the Type II **CBD** from Pseudomonas fluorescens subsp. cellulosa xylanase A (XYLACBD) and cellulase E (CELECBD) were expressed as individual entities or fused to the catalytic domain of a Clostridium thermocellum endoglucanase (EGE). The two **CBDs** exhibited similar Ka values for bacterial microcrystalline cellulose (CELECBD, 1.62x10(6) M-1; XYLACBD, 1.83x10(6) M-1) and acid-swollen cellulose (CELECBD, 1.66x10(6) M-1; XYLACBD, 1.73x10(6) M-1). NMR spectra of XYLACBD titrated with cello-oligosaccharides showed that the environment of three tryptophan residues was affected when the **CBD** bound cellohexaose, cellopentaose or cellotetraose. The Ka values of the XYLACBD for C6, C5 and C4 cello-oligosaccharides were estimated to be 3.3x10(2), 1.4x10(2) and 4.0x10(1) M-1 respectively, suggesting that the **CBD** can accommodate at least six glucose molecules and has a much higher affinity for insoluble cellulose than soluble oligosaccharides. Fusion of either the CELECBD or XYLACBD to the catalytic domain of EGE potentiated the activity of the enzyme against insoluble forms of cellulose but not against carboxymethylcellulose. The increase in cellulase activity was not observed when the **CBDs** were incubated with the catalytic domain of either EGE or XYLA, with insoluble cellulose and a cellulose/hemicellulose complex respectively as the substrates. Pseudomonas **CBDs** did not induce the extension of isolated plant cell walls nor weaken cellulose paper strips in the same way as a class of plant cell wall proteins called expansins. The XYLACBD and CELECBD did not release small particles from the surface of cotton. The significance of these results in relation to the mode of action of Type II **CBDs** is discussed.

L3 ANSWER 73 OF 224 MEDLINE on STN
ACCESSION NUMBER: 1998053843 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9393694
TITLE: Cloning, sequencing, and expression of the gene encoding Clostridium paraputrificum chitinase ChiB and analysis of the functions of novel cadherin-like domains and a chitin-binding domain.

AUTHOR: Morimoto K; Karita S; Kimura T; Sakka K; Ohmiya K
CORPORATE SOURCE: Faculty of Bioresources, Mie University, Tsu, Japan.
SOURCE: Journal of bacteriology, (1997 Dec) Vol. 179, No. 23, pp. 7306-14.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB001874
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 16 Jan 1998
Last Updated on STN: 16 Jan 1998
Entered Medline: 30 Dec 1997

AB The Clostridium paraputrificum chiB gene, encoding chitinase B (ChiB), consists of an open reading frame of 2,493 nucleotides and encodes 831 amino acids with a deduced molecular weight of 90,020. The deduced ChiB is a modular enzyme composed of a family 18 catalytic domain responsible for chitinase activity, two reiterated domains of unknown function, and a chitin-binding domain (**CBD**). The reiterated domains are similar to the repeating units of cadherin proteins but not to fibronectin type III domains, and therefore they are referred to as cadherin-like domains. ChiB was purified from the periplasm fraction of Escherichia coli harboring the chiB gene. The molecular weight of the purified ChiB (87,000) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, was in good agreement with the value (86,578) calculated from the deduced amino acid sequence excluding the signal peptide. ChiB was active toward chitin from crab shells, colloidal chitin, glycol chitin, and 4-methylumbelliferyl beta-D-N,N'-diacetylchitobioside [4-MU-(GlcNAc)2]. The pH and temperature optima of the enzyme were 6.0 and 45 degrees C, respectively. The Km and Vmax values for 4-MU-(GlcNAc)2 were estimated to be 6.3 microM and 46 micromol/min/mg, respectively. SDS-PAGE, zymogram, and Western blot analyses using antiserum raised against purified ChiB suggested that ChiB was one of the major chitinase species in the culture supernatant of C. paraputrificum. Deletion analysis showed clearly that the **CBD** of ChiB plays an important role in hydrolysis of native chitin but not processed chitin such as colloidal chitin.

L3 ANSWER 74 OF 224 MEDLINE on STN
ACCESSION NUMBER: 97284479 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9139893
TITLE: Role of scaffolding protein CipC of Clostridium cellulolyticum in cellulose degradation.
AUTHOR: Pages S; Gal L; Belaich A; Gaudin C; Tardif C; Belaich J P
CORPORATE SOURCE: Bioenergetique et Ingenierie des Proteines, Centre National de la Recherche Scientifique, IBSM-IFR1, Marseille, France.
SOURCE: Journal of bacteriology, (1997 May) Vol. 179, No. 9, pp. 2810-6.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 9 Jun 1997
Last Updated on STN: 9 Jun 1997
Entered Medline: 29 May 1997

AB The role of a miniscaffolding protein, miniCipC1, forming part of Clostridium cellulolyticum scaffolding protein CipC in insoluble cellulose degradation was investigated. The parameters of the binding of miniCipC1, which contains a family III cellulose-binding domain (**CBD**), a hydrophilic domain, and a cohesin domain, to four insoluble celluloses

were determined. At saturating concentrations, about 8.2 micromol of protein was bound per g of bacterial microcrystalline cellulose, while Avicel, colloidal Avicel, and phosphoric acid-swollen cellulose bound 0.28, 0.38, and 0.55 micromol of miniCipC1 per g, respectively. The dissociation constants measured varied between 1.3 x 10(-7) and 1.5 x 10(-8) M. These results are discussed with regard to the properties of the various substrates. The synergistic action of miniCipC1 and two forms of endoglucanase CelA (with and without the dockerin domain [CelA2 and CelA3, respectively]) in cellulose degradation was also studied. Although only CelA2 interacted with miniCipC1 (K(d), 7 x 10(-9) M), nonhydrolytic miniCipC1 enhanced the activities of endoglucanases CelA2 and CelA3 with all of the insoluble substrates tested. This finding shows that miniCipC1 plays two roles: it increases the enzyme concentration on the cellulose surface and enhances the accessibility of the enzyme to the substrate by modifying the structure of the cellulose, leading to an increased available cellulose surface area. In addition, the data obtained with a hybrid protein, CelA3-**CBD**(CipC), which was more active towards all of the insoluble substrates tested confirm that the **CBD** of the scaffolding protein plays an essential role in cellulose degradation.

L3 ANSWER 75 OF 224 MEDLINE on STN
ACCESSION NUMBER: 97332540 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9188774
TITLE: The Ca2+/calmodulin binding domain of the Ca2+-ATPase linked to the Na+,K+-ATPase alters transport stoichiometry.
AUTHOR: Zhao J; Vasilets L A; Yoshimura S H; Gu Q; Ishii T; Takeyasu K; Schwarz W
CORPORATE SOURCE: Max-Planck Institut fur Biophysik, Frankfurt/Main, Germany.
SOURCE: FEBS letters, (1997 May 26) Vol. 408, No. 3, pp. 271-5. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 21 Jul 1997
Last Updated on STN: 21 Jul 1997
Entered Medline: 10 Jul 1997

AB Using Xenopus oocytes as an expression system, we have investigated ion-transport and ouabain-binding properties of a chimeric ATPase (alpha1-**CBD**; Ishii and Takeyasu (1995) EMBO J. 14, 58-67) formed by the alpha1-subunit of chicken Na+,K(+)-ATPase (alpha1) and the calmodulin binding domain (**CBD**) of the rat plasma membrane Ca2(+)-ATPase. alpha1-**CBD** can be expressed and transported to the oocyte plasma membrane without the beta-subunit, and shows ouabain binding. In contrast to ouabain binding, this chimera requires the beta-subunit for its cation (Na+ and K+) transport activity. alpha1-**CBD** exhibits an altered stoichiometry of Na(+)-K+ exchange. A detailed analysis of 22Na+ efflux, 86Rb+ uptake, pump current and ouabain binding suggests that the chimeric molecule can operate in an electrically silent 2Na(+)-2K+ exchange mode and, with much lower probability, in its normal 3Na(+)-2K+ exchange mode.

L3 ANSWER 76 OF 224 MEDLINE on STN
ACCESSION NUMBER: 95362661 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7635821
TITLE: Comparison of a fungal (family I) and bacterial (family II) cellulose-binding domain.
AUTHOR: Tomme P; Driver D P; Amandoron E A; Miller R C Jr; Antony R; Warren J; Kilburn D G
CORPORATE SOURCE: Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada.
SOURCE: Journal of bacteriology, (1995 Aug) Vol. 177, No. 15, pp. 4356-63.

Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 21 Sep 1995
Last Updated on STN: 21 Sep 1995
Entered Medline: 13 Sep 1995

AB A family II cellulose-binding domain (**CBD**) of an exoglucanase/xylanase (Cex) from the bacterium Cellulomonas fimi was replaced with the family I **CBD** of cellobiohydrolase I (CbhI) from the fungus Trichoderma reesei. Expression of the hybrid gene in Escherichia coli yielded up to 50 mg of the hybrid protein, CexCBDCbhI, per liter of culture supernatant. The hybrid was purified to homogeneity by affinity chromatography on cellulose. The relative association constants (Kr) for the binding of Cex, CexCBDCbhI, the catalytic domain of Cex (p33), and CbhI to bacterial microcrystalline cellulose (BMCC) were 14.9, 7.8, 0.8, and 10.6 liters g-1, respectively. Cex and CexCBDCbhI had similar substrate specificities and similar activities on crystalline and amorphous cellulose. Both released predominantly cellobiose and cellotriose from amorphous cellulose. CexCBDCbhI was two to three times less active than Cex on BMCC, but significantly more active than Cex on soluble cellulose and on xylan. Unlike Cex, the hybrid protein neither bound to alpha-chitin nor released small particles from dewaxed cotton fibers.

L3 ANSWER 77 OF 224 MEDLINE on STN
ACCESSION NUMBER: 95374012 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7646033
TITLE: Expression, purification, and characterization of the cellulose-binding domain of the scaffoldin subunit from the cellulosome of Clostridium thermocellum.
AUTHOR: Morag E; Lapidot A; Govorko D; Lamed R; Wilchek M; Bayer E A; Shoham Y
CORPORATE SOURCE: Department of Biophysics, Weizmann Institute of Science, Rehovot, Israel.
SOURCE: Applied and environmental microbiology, (1995 May) Vol. 61, No. 5, pp. 1980-6.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 30 Sep 1995
Last Updated on STN: 30 Sep 1995
Entered Medline: 19 Sep 1995

AB The major cellulose-binding domain (**CBD**) from the cellulosome of Clostridium thermocellum YS was cloned and overexpressed in Escherichia coli. The expressed protein was purified efficiently by a modification of a novel procedure termed affinity digestion. The properties of the purified polypeptide were compared with those of a related **CBD** derived from a cellulosome-like complex of a similar (but mesophilic) clostridial species, Clostridium cellulovorans. The binding properties of the two proteins with their common substrate were found to be very similar. Despite the similarity in the amino acid sequences of the two **CBDs**, polyclonal antibodies raised against the **CBD** from C. thermocellum failed to interact with the protein from C. cellulovorans. Chemical modification of the single cysteine of the **CBD** had little effect on the binding to cellulose. Biotinylation of this cysteine allowed the efficient binding of avidin to cellulose, and the resultant matrix is appropriate for use as a universal affinity system.

L3 ANSWER 78 OF 224 MEDLINE on STN
ACCESSION NUMBER: 95302955 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7783614
TITLE: Identification of a novel cellulose-binding domain within the multidomain 120 kDa xylanase XynA of the hyperthermophilic bacterium Thermotoga maritima.
AUTHOR: Winterhalter C; Heinrich P; Candussio A; Wich G; Liebl W
CORPORATE SOURCE: Lehrstuhl fur Mikrobiologie, Technische Universitat, Munchen, Germany.
SOURCE: Molecular microbiology, (1995 Feb) Vol. 15, No. 3, pp. 431-44.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Z46264
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 26 Jul 1995
Last Updated on STN: 26 Jul 1995
Entered Medline: 19 Jul 1995

AB A segment of Thermotoga maritima strain MSB8 chromosomal DNA was isolated which encodes an endo-1,4-beta-D-xylanase, and the nucleotide sequence of the xylanase gene, designated xynA, was determined. With a half-life of about 40 min at 90 degrees C at the optimal pH of 6.2, purified recombinant XynA is one of the most thermostable xylanases known. XynA is a 1059-amino-acid (approximately 120 kDa) modular enzyme composed of an N-terminal signal peptide and five domains, in the order A1-A2-B-C1-C2. By comparison with other xylanases of family 10 of glycosyl hydrolases, the central approximately 340-amino-acid part (domain B) of XynA represents the catalytic domain. The N-terminal approximately 150-amino-acid repeated domains (A1-A2) have no significant similarity to the C-terminal approximately 170-amino-acid repeated domains (C1-C2). Cellulose-binding studies with truncated XynA derivatives and hybrid proteins indicated that the C-terminal repeated domains mediate the binding of XynA to microcrystalline cellulose and that C2 alone can also promote cellulose binding. C1 and C2 did not share amino acid sequence similarity with any other known cellulose-binding domain (**CBD**) and thus are **CBDs** of a novel type. Structurally related protein segments which are probably also **CBDs** were found in other multidomain xylanolytic enzymes. Deletion of the N-terminal repeated domains or of all the non-catalytic domains resulted in substantially reduced thermostability while a truncated xylanase derivative lacking the C-terminal tandem repeat was as thermostable as the full-length enzyme.(ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 79 OF 224 MEDLINE on STN
ACCESSION NUMBER: 93372174 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8364096
TITLE: An attempt to substitute the cell binding domain of human fibronectin in lambda phage J protein: computer design and expression.
AUTHOR: Shibata K; Mita T; Nakamura H; Yamashiro K; Gotoh S; Hiranuma K; Higashi K; Hirano H
CORPORATE SOURCE: Department of Molecular Biology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan.
SOURCE: Biochimie, (1993) Vol. 75, No. 6, pp. 459-65.
Journal code: 1264604. ISSN: 0300-9084.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-A03213
ENTRY MONTH: 199310
ENTRY DATE: Entered STN: 22 Oct 1993
Last Updated on STN: 22 Oct 1993
Entered Medline: 7 Oct 1993

AB We superimposed hydropathic indexes of the human fibronectin cell binding domain (**CBD**) on the lambda phage J protein by computer, and substituted 22 amino acids from the fibronectin **CBD** for a part of the lambda phage J protein. The fibronectin cell binding domain -Arg-Gly-Asp-Ser- (-RGDS-) localizes at the junction of hydrophobicity and hydrophilicity. We selected a similar hydrophobic-to-hydrophilic junction in the J protein region for substitution. This junction corresponds to 150 bp of the PstI fragment of J protein cDNA. We synthesized 150 bp of the relevant PstI fragment that includes the cell binding domain region. The region was then constructed by serial cloning as an expression vector, pJCBD. The vector pJCBD expressed the fused protein named JCBD (M(r) 32 kDa) in E coli XL1-BLUE. The expressed JCBD protein was identified by Western blot analysis in the extract of the pJCBD carrying bacterial lysate using both rabbit anti-lambda phage antiserum and anti-**CBD** of fibronectin antibody. The JCBD protein appeared to recognize retinoblast cell membrane RGDS-directed receptors, detected by enzyme-linked immuno-sorbent assay and also by binding competition assay with synthetic pentapeptides, Gly-Arg-Gly-Asp-Ser (GRGDS) and Gly-Arg-Gly-Glu-Ser (GRGES). The former competitor inhibited completely fibronectin **CBD**-dependent binding activity of JCBD, the latter had no inhibitory activity. These results suggest that certain functional proteins engineered by computer design between human fibronectin cell binding domain and lambda phage J protein can be produced.

L3 ANSWER 80 OF 224 MEDLINE on STN
ACCESSION NUMBER: 94269191 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8208848
TITLE: The N-terminal cysteine-rich domain of tobacco class I chitinase is essential for chitin binding but not for catalytic or antifungal activity.
AUTHOR: Iseli B; Boller T; Neuhaus J M
CORPORATE SOURCE: Botanisches Institut, Universitat Basel, Switzerland.
SOURCE: Plant physiology, (1993 Sep) Vol. 103, No. 1, pp. 221-6.
Journal code: 0401224. ISSN: 0032-0889.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 21 Jul 1994
Last Updated on STN: 3 Mar 2000
Entered Medline: 14 Jul 1994

AB The vacuolar chitinases of class I possess an N-terminal cysteine-rich domain homologous to hevein and chitin-binding lectins such as wheat germ agglutinin and Urtica dioica lectin. To investigate the significance of this domain for the biochemical and functional characteristics of chitinase, chimeric genes encoding the basic chitinase A of tobacco (Nicotiana tabacum) with and without this domain were constructed and constitutively expressed in transgenic Nicotiana sylvestris. The chitinases were subsequently isolated and purified to homogeneity from the transgenic plants. Chromatography on colloidal chitin revealed that only the form with the N-terminal domain, and not the one without it, had chitin-binding properties, demonstrating directly that the domain is a chitin-binding domain (**CBD**). Under standard assay conditions with radioactive colloidal chitin, both forms of chitinase had approximately the same catalytic activity. However, kinetic analysis demonstrated that the enzyme without **CBD** had a considerably

lower apparent affinity for its substrate. The pH and temperature optima of the two chitinases were similar, but the form with the **CBD** had an approximately 3-fold higher activation energy and retained a higher activity at low pH values. Both chitinases were capable of inhibiting growth of Trichoderma viride, although the form with the **CBD** was about three times more effective than the one without it. Thus, the **CBD** is not necessary for catalytic or antifungal activity of chitinase.

L3 ANSWER 81 OF 224 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2006) on STN DUPLICATE 43

ACCESSION NUMBER: 2002:44247 AGRICOLA
DOCUMENT NUMBER: IND23274327
TITLE: Design of a fungal cellulose-binding domain for PCR amplification and expression in E. coli.
AUTHOR(S): Amouri, B.; Fradi, N.; Gargouri, A.
AVAILABILITY: DNAL (QR53.B56)
SOURCE: Biotechnology letters, Nov 2001. Vol. 23, No. 22. p. 1883-1888
CODEN: BILED3; ISSN: 0141-5492
NOTE: Includes references
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Article
FILE SEGMENT: Non-US
LANGUAGE: English

AB A new fungal cellulose binding domain (**CBD**) from Stachybotris sp. has been cloned. Multiple sequence alignment of the **CBD** from 34 fungi shows highest sequence identity at the ends of the domains. The two primers from these regions were amplified by PCR giving a 120-bp product. Two of these, from Trichoderma sp. and Stachybotris sp. were subsequently cloned, sequenced and confirmed to be of the **CBD** family. The **CBD** from Stachybotris sp. was expressed in E. coli fused to g3p of the M13 phage and with a c-myc tag. The secreted **fusion protein** adsorbed on acid-swollen cellulose thereby confirming its functionality.

L3 ANSWER 82 OF 224 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2006) on STN DUPLICATE 49

ACCESSION NUMBER: 2000:33472 AGRICOLA
DOCUMENT NUMBER: IND22043465
TITLE: Novel methodology for enzymatic removal of atrazine from water by **CBD-fusion protein** immobilized on cellulose.
AUTHOR(S): Kauffmann, C.; Shoseyov, O.; Shpigel, E.; Bayer, E.A.; Lamed, R.; Shoham, Y.; Mandelbaum, R.T.
CORPORATE SOURCE: Hebrew University of Jerusalem, Rehovot.
AVAILABILITY: DNAL (TD420.A1E5)
SOURCE: Environmental science & technology, Apr 1, 2000. Vol. 34, No. 7. p. 1292-1296
Publisher: Washington, D.C. : American Chemical Society.
CODEN: ESTHAG; ISSN: 0013-936X
NOTE: Includes references
PUB. COUNTRY: District of Columbia; United States
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

L3 ANSWER 83 OF 224 AGRICOLA Compiled and distributed by the National

 DUPLICATE 81

ACCESSION NUMBER: 96:175 AGRICOLA
DOCUMENT NUMBER: IND20487971
TITLE: Purification and processing of cellulose-binding domain-alkaline phosphatase **fusion proteins.**
AUTHOR(S): Greenwood, J.M.; Gilkes, N.R.; Miller, R.C. Jr; Kilburn, D.G.; Antony, R.; Warren, J.
CORPORATE SOURCE: Cell Therapeutics Inc., Seattle, WA.
AVAILABILITY: DNAL (381 J8224)
SOURCE: Biotechnology and bioengineering, Dec 1994. Vol. 44, No. 11. p. 1295-1305
Publisher: New York : John Wiley & Sons, Inc.
CODEN: BIBIAU; ISSN: 0006-3592
NOTE: Includes references
PUB. COUNTRY: New York (State); United States
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB Fusion of the leader peptide and the cellulose-binding domain (**CBD**) of endoglucanase A (CenA) from Cellulomonas fimi, with or without linker sequences, to the N-terminus of alkaline phosphatase (PhoA) from Escherichia cold leads to the accumulation of significant amounts of the **CBD**-PhqA **fusion proteins** in the supernatants of E. coli cultures. The **fusion proteins** can be purified from the supernatants by affinity chromatography on cellulose. The **fusion proteins** can be desorbed from the cellulose with water or guanidine-HCl. If the sequence IEGR is present between the **CBD** and PhoA, the **CBD** can be cleaved from the PhoA with factor Xa. The efficiency of hydrolysis by factor Xa is strongly influenced by the amino acids on either side of the IEGR sequence. The **CBD** released by factor Xa is removed by adsorption to cellulose. A nonspecific protease from C. fimi, which hydrolyzes native CenA between the **CBD** and the catalytic domain, may be useful for removing the **CBD** from some **fusion proteins.**

L3 ANSWER 84 OF 224 AGRICOLA DUPLICATE 85

ACCESSION NUMBER: 93:61769 AGRICOLA
DOCUMENT NUMBER: IND93040314
TITLE: Proteins designed for adherence to cellulose.
AUTHOR(S): Ong, E.; Greenwood, J.M.; Gilkes, N.R.; Miller, R.C. Jr; Warren, R.A.J.; Kilburn, D.G.
CORPORATE SOURCE: University of British Columbia, Vancouver, British Columbia, Canada
AVAILABILITY: DNAL (QD1.A45)
SOURCE: ACS Symposium series - American Chemical Society, 1993. No. 516. p. 185-194
Publisher: Washington, D.C. : The Society.
CODEN: ACSMC8; ISSN: 0097-6156
NOTE: In the series analytic: Biocatalyst design for stability and specificity / edited by M.E. Himmel and G. Georgiou.
Includes references.
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB Molecular genetic techniques have been used to produce **fusion proteins** containing the cellulose-binding domain (**CBD**)

of the cellulases CenA or Cex from the bacterium Cellulomonas fimi. **CBD**(CenA) is at the N-terminus of CenA, whereas **CBD**(Cex) is at the C-terminus of Cex. Using appropriate cloning vectors, a **CBD** can be fused either to the N- or the C-terminus of a desired protein, an advantage if fusion at one terminus but not the other inactivates the heterologous protein. Vectors can be modified further to allow construction of **fusion proteins** containing sites for proteolytic removal of the cellulose-binding domain. The **fusion proteins** bind tightly to cellulose under normal physiological conditions but can be easily eluted with water or elevated pH, or digested with protease in situ, allowing purification almost to homogeneity in a single step. Under appropriate conditions, adsorption of hybrid enzymes to cellulose is effectively irreversible. **CBDs** thus provide a generic system for enzyme immobilization on an inexpensive, convenient matrix.

L3 ANSWER 85 OF 224 DRUGU COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2006-00450 DRUGU P
TITLE: Analysing the effect of novel therapies on cytokine expression in experimental arthritis.
AUTHOR: Williams R O; Inglis J J; Simelyte E; Criado G; Sumariwalla P F
CORPORATE SOURCE: Univ.London
LOCATION: London, U.K.
SOURCE: Int.J.Exp.Pathol. (86, No. 5, 267-78, 2005) 6 Fig. 60 Ref.
CODEN: IJEPEI ISSN: 0959-9673
AVAIL. OF DOC.: Kennedy Institute of Rheumatology, Division, Imperial College London, 1 Aspenlea Road, London W6 8LH, England. (e-mail: richard.o.williams@imperial.ac.uk).
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 2006-00450 DRUGU P
AB The effect of novel therapies on cytokine expression in experimental rheumatoid arthritis (RA) is reviewed. Subjects covered are the impact of TNF-alpha blockade on arthritis, the need to quantify cytokine expression in vivo, detection of cytokines in situ, detection of cytokines in synovial membrane cell cultures, spleen and lymph node cell assays and measurement of cytokine mRNA. It is clear that many useful techniques exist for the detection and quantification of cytokines in animal models of arthritis and all of them have the potential to increase our knowledge and understanding not only of disease processes but also of how drugs affect these processes. In addition, developments in genomic and proteomic array technologies will enable much more comprehensive and nonhypothesis-driven studies of disease and the response to therapy.
ABEX The collagen-induced arthritis (CIA) model exhibits many clinical similarities to human RA, and both the diseases exhibit similar patterns of synovitis, pannus formation, cartilage and bone erosion, fibrosis and loss of joint function. A number of anti-TNF-alpha mAb have now been shown in clinical trials to be effective in patients with severe RA, refractory to existing disease modifying drugs including infliximab. Various TNF receptor-based biologics have also been tested and shown to be effective, including dimeric p75 TNF receptor-Fc **fusion protein**, etanercept, p55 TNF receptor-Fc **fusion protein**, lenercept and pegylated monomeric p55 TNF-receptor. Anti-arthritic effect of cannabidiol (**CBD**) is partially attributed to reduction in bioactive TNF-alpha output from knee synovial cells of **CBD**-treated mice. Salbutamol has been reported to reduce the TNF-alpha and IL-12 levels by elevating cAMP, the secondary messenger in cells. All cAMP-elevating agents (rolipram, forskolin (colforsin), PGE2, 8-bromo-cAMP or cholera toxin) dose-dependently suppressed IFN-gamma production. Abeta T cells, but not gammadelta T

cells, have been shown to be involved in the development of RA. In CIA, the synovial mRNA expression levels for IL-6 and IL-1beta are reduced after prednisolone. I.v. or intra-articular injections of Avenue (viral IL-10), a replication-deficient adenovirus encoding vIL-10, is associated with delayed synovial expression of proinflammatory cytokines IL-2 and IL-1beta mRNA in response to CII immunization as determined by RPA. The levels of cytokine expression are decreased in mice treated with mIL-18BP. (ND/CW)

L3 ANSWER 86 OF 224 JICST-EPlus COPYRIGHT 2006 JST on STN DUPLICATE 68
ACCESSION NUMBER: 980437770 JICST-EPlus
TITLE: A Novel Drug Delivery System with A Collagen-Binding Domain derived from C. Histolyticum Collagenase.
AUTHOR: NISHI NOZOMU; MATSUSHITA OSAMU
CORPORATE SOURCE: Kagawa Med. Sch.
SOURCE: Connect Tissue, (1998) vol. 30, no. 1, pp. 37-42. Journal Code: G0168B (Fig. 6, Ref. 7)
ISSN: 0916-572X
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: Japanese
STATUS: New
AB The extracellular matrix (ECM) is an attractive target for localizing exogenous growth factors and other peptide signaling molecules as therapeutic agents. We have produced **fusion proteins** consisting of growth factor moieties and a collagen-binding domain (**CBD**) derived from a bacterial collagenase which we expected to act as an anchor to the collagen fibrils in vivo. The **fusion proteins** carrying the epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) at the N-terminal of **CBD** (CBEGF & CBFGF) tightly bound to insoluble collagen and stimulated the growth of cultured fibroblasts as much as the unfused counterparts. CBEGF, when injected subcutaneously into mice, remained at the sites of injection for up to 10 days, but EGF was not detectable 24 h after injection. Although CBEGF did not exert a growth promoting effect in vivo, CBFGF, but not bFGF, strongly stimulated the DNA synthesis in stromal cells at 5 days and 7 days after injection. **CBD** may be used as an anchoring unit to produce **fusion proteins** which are nondiffusible and long-lasting in vivo. (author abst.)

L3 ANSWER 87 OF 224 JICST-EPlus COPYRIGHT 2006 JST on STN DUPLICATE 72
ACCESSION NUMBER: 980016949 JICST-EPlus
TITLE: Purification of the Ruminococcus albus Endoglucanase IV Using a Cellulose-Binding Domain as an Affinity Tag.
AUTHOR: KARITA S; KIMURA T; SAKKA K; OHMIYA K
CORPORATE SOURCE: Mie Univ., Tsu, JPN
SOURCE: J Ferment Bioeng, (1997) vol. 84, no. 4, pp. 354-357. Journal Code: G0535B (Fig. 2, Tbl. 1, Ref. 17)
CODEN: JFBIEX; ISSN: 0922-338X
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Short Communication
LANGUAGE: English
STATUS: New
AB The gene encoding the single cellulose-binding domain II (**CBD** II) of Clostridium stercorarium xylanase A was fused to the egIVgene encoding endoglucanase IV (EGIV) from Ruminococcus albus. The **fusion protein** (EGIV+CBDII) expressed in Escherichia coli can be readily purified from the cell-free extract of E. coli in a single step using the affinity of **CBD** to cellulose. The purified enzyme was cleaved into two moieties, i.e. the catalytic domain and **CBD**, at a specific site in the linker region by partial digestion with trypsin at 4.DEG.C.. This result indicates that this **CBD** belonging to family VI of **CBD** families can be used as an

affinity tag for purification of the recombinant protein. (author abst.)

L3 ANSWER 88 OF 224 JICST-EPlus COPYRIGHT 2006 JST on STN DUPLICATE 75
ACCESSION NUMBER: 960627736 JICST-EPlus
TITLE: Cellulose-Binding Domains Confer an Enhanced Activity against Insoluble Cellulose to Ruminococcus albus Endoglucanase IV.
AUTHOR: KARITA S; SAKKA K; OHMIYA K
CORPORATE SOURCE: Mie Univ., Tsu, JPN
SOURCE: J Ferment Bioeng, (1996) vol. 81, no. 6, pp. 553-556. Journal Code: G0535B (Fig. 3, Tbl. 1, Ref. 24) CODEN: JFBIEX; ISSN: 0922-338X
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Short Communication
LANGUAGE: English
STATUS: New
AB The gene encoding cellulose-binding domains (**CBDs**) from Clostridium stercorarium xylanase A was joined to the egIV gene encoding endoglucanase IV (EGIV) from Ruminococcus albus. The hybrid protein (EGIV + **CBD**) expressed from this fusion gene in Escherichia coli acquired the ability to adsorb onto insoluble celluloses such as ball-milled cellulose (BMC). EGIV + **CBD** was more active toward BMC at low concentrations than the parental enzyme, EGIV, although there was no difference in the catalytic properties between them toward carboxymethyl cellulose. This result indicates that the addition of the **CBDs** to EGIV enhances enzyme activity on the insoluble cellulose by concentrating the catalytic domain on the substrate surface. (author abst.)

L3 ANSWER 89 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN DUPLICATE
ACCESSION NUMBER: 2003:36917591 BIOTECHNO
TITLE: Obtaining purified p24 gag protein of HIV-1 by using a new prokaryotic expressive vector
AUTHOR: Wang Z.-C.; Teng Z.-P.; Zhang X.-G.; Guo X.-C.; Yuan J.-M.; Zeng Y.
CORPORATE SOURCE: X.-G. Zhang, Institute of Virology, Chinese Acad. of Preventive Medicine, Beijing 100052, China. E-mail: XGZhang01@yahoo.com
SOURCE: Chinese Journal of Microbiology and Immunology, (30 MAY 2003), 23/5 (375-379), 13 reference(s) CODEN: ZWMZDP ISSN: 0254-5101
DOCUMENT TYPE: Journal; Article
COUNTRY: China
LANGUAGE: Chinese
SUMMARY LANGUAGE: English; Chinese
AN 2003:36917591 BIOTECHNO
AB Objective: To obtaining purified p24 gag protein of HIV-1 by a new prokaryotic expressive vector. Methods: p24 fragment was amplified by PCR from the HIV-1 HXB2 cDNA template and cloned into the expressive plasmid pTXB1 for constructing the recombinant plasmid pTXB-p24. Purified p24 was obtained by affinity column (chitin beads). Molecular weight and purity of p24 were analyzed with SDS-PAGE and HPLC, Antigenity was tested with Western blot and colloid gold technique. Results: Under the control of phage T7 promoter leading to production of **fusion protein** p24-Intein-**CBD** in E. coli, stable expression quantity was above 12%. The purity of p24 was more than 96% with HPLC, and it reacted with serum samples from HIV-1 infected subjects when tested by Western blot and colloid gold technique. Conclusion: Obtaining purified non-fusion p24 gag protein of HIV-1 with a new prokaryotic expressive vector, may reach a purity more than 96%.

L3 ANSWER 90 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN

DUPLICATE
ACCESSION NUMBER: 2002:34556786 BIOTECHNO
TITLE: Construction of engineered yeast with the ability of binding to cellulose
AUTHOR: Nam J.-M.; Fujita Y.; Arai T.; Kondo A.; Morikawa Y.; Okada H.; Ueda M.; Tanaka A.
CORPORATE SOURCE: M. Ueda, Department of Synthetic Chemistry, Graduate School of Engineering, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan.
E-mail: miueda@sbchem.kyoto-u.ac.jp
SOURCE: Journal of Molecular Catalysis - B Enzymatic, (07 JUN 2002), 17/3-5 (197-202), 14 reference(s)
CODEN: JMCEF8 ISSN: 1381-1177
PUBLISHER ITEM IDENT.: S1381117702000280
DOCUMENT TYPE: Journal; Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2002:34556786 BIOTECHNO
AB The genes encoding cellulose binding domain (**CBD**) from cellobiohydrolase I (CBHI) and cellobiohydrolase II (CBHII) of the filamentous fungus Trichoderma reesei were expressed on the cell surface of the yeast Saccharomyces cerevisiae by cell surface engineering. The **CBD** genes were fused to the gene encoding the Rhizopus oryzae glucoamylase secretion signal sequence, and expressed under the control of the glyceraldehydes-3-phosphate dehydrogenase (GAPDH) promoter. Each of **CBDs** was successfully displayed on the yeast cell surface by fusing their genes to the gene encoding the 3'-half of α-agglutinin of S. cerevisiae having a glycosylphosphatidylinositol anchor attachment signal. Tandemly aligned CBHI (CBD1) and CBHII (CBD2) fusion gene was also constructed to display simultaneously both **CBDs** on the cell surface of S. cerevisiae. Binding affinity of the **CBD** -displaying yeast cells to a cellulose substrate was similar between the CBD1- and CBD2-displaying yeast cells. However, the cells displaying the **fusion protein** of CBD1 and CBD2 showed much higher binding affinity to cellulose than either of the single **CBD** -displaying yeast cells. The binding affinity of the cells was increased by treating the cellulose with phosphoric acid.

L3 ANSWER 91 OF 224 BIOTECHNO
DUPLICATE
ACCESSION NUMBER: 2002:35094474 BIOTECHNO
TITLE: Stabilization of horseradish peroxidase in aqueous-organic media by immobilization onto cellulose using a cellulose-binding-domain
AUTHOR: Fishman A.; Levy I.; Cogan U.; Shoseyov O.
CORPORATE SOURCE: A. Fishman, IMI (TAMI) Institute for R and D, P.O. Box 10140, Haifa 26111, Israel.
E-mail: ayelet@tami-imi.co.il
SOURCE: Journal of Molecular Catalysis B: Enzymatic, (13 SEP 2002), 18/1-3 (121-131), 64 reference(s)
CODEN: JMCEF8 ISSN: 1381-1177
PUBLISHER ITEM IDENT.: S1381117702000759
DOCUMENT TYPE: Journal; Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2002:35094474 BIOTECHNO
AB A fused protein consisting of a cellulose-binding domain (**CBD**) and horseradish peroxidase (HRP) was bound to cellulose beads and evaluated in aqueous-organic solvent systems. The **CBD**-HRP **fusion protein** containing two different

functionalities, a catalytic domain and a binding domain, preserved both capabilities in this non-conventional environment. A six-fold increase in the half-life of the enzyme in buffer resulted from immobilization onto cellulose via **CBD**. The immobilized enzyme was also more stable than the native enzyme in increasing concentrations of acetone (0-92%). There was a general decrease in activity as the solvent concentration in the mixture increased (in all solvent types: THF, acetone, acetonitrile and ethanol). However, the immobilized enzyme was at all times more active than the soluble enzyme forms. The thermostability of the enzyme in buffer, at 40-60°C, was also improved by immobilization. The soluble **CBD**-HRP **fusion protein** exhibited greater stability (both to organic solvents and temperature), but lower activity, in comparison with the native HRP. This work demonstrates for the first time the use of a cellulose-bound **CBD**-enzyme as a catalyst in aqueous-organic solvent media.

L3 ANSWER 92 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE

ACCESSION NUMBER: 2001:34209137 BIOTECHNO
TITLE: Modeling of growth and energy metabolism of Pichia pastoris producing a **fusion protein**
AUTHOR: Jahic M.; Rotticci-Mulder J.C.; Martinelle M.; Hult K.; Enfors S.-O.
CORPORATE SOURCE: S.-O. Enfors, Department of Biotechnology, Royal Institute of Technology, Roslagstullbacken 21, 10691 Stockholm, Sweden.
E-mail: olle@biochem.kth.se
SOURCE: Bioprocess and Biosystems Engineering, (2001), 24/6 (385-393), 23 reference(s)
CODEN: BBEIBV ISSN: 1615-7591
DOCUMENT TYPE: Journal; Article
COUNTRY: Germany, Federal Republic of
LANGUAGE: English
SUMMARY LANGUAGE: English

AN 2001:34209137 BIOTECHNO

AB A **fusion protein** composed of a cellulose binding domain from Neocallimastix patriciarum cellulase A and Candida antarctica lipase B (**CBD**-lipase) was produced by Pichia pastoris methanol utilization plus phenotype in high cell-density cultures. The genes expressing **CBD**-lipase were fused to the alpha-factor secretion signal sequence of Saccharomyces cerevisiae and placed under the control of the alcohol oxidase gene (AOX1) promoter. To control the repression and induction of AOX1 and oxygen demand at high cell density, a four-stage process was used. Batch growth on glycerol was used in the first step to provide biomass (28 g L.sup.-.sup.1) while product formation was prevented due to repression of the AOX1. The second stage was exponential fed-batch growth on glycerol, which caused a slight increase of the enzyme alcohol oxidase activity due to derepression of the AOX1. This procedure resulted in smooth transition to exponential fed-batch growth on methanol, the third stage, in which the AOX1 was strongly induced. The fourth stage was constant fed-batch growth on methanol used to control the oxygen demand at the high cell density. A kinetic model was developed that could predict biomass growth and oxygen consumption in processes with and without oxygen-enriched air. With oxygen enrichment to 34% O.sub.2 in the inlet air the methanol feed rate could be increased by 50% and this resulted in 14% higher final cell density (from 140 to 160 g L.sup.-.sup.1 cell dry weight). The increased methanol feed rate resulted in a proportionally increased specific rate of product secretion to the medium. After an initial decrease, the synthesis capacity of the cell was kept constant throughout the cultivation, which made the product concentration increase almost constantly during the process. The kinetic model also describes how the

low maintenance demand of P. pastoris compared with E. coli enables this organism to grow to such high cell densities.

L3 ANSWER 93 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1999:29209975 BIOTECHNO
TITLE: Cellulose as an inert matrix for presenting cytokines to target cells: Production and properties of a stem cell factor-cellulose-binding domain **fusion protein**
AUTHOR: Doheny J.G.; Jervis E.J.; Guarna M.M.; Humphries R.K.; Warren R.A.J.; Kilburn D.G.
CORPORATE SOURCE: D.G. Kilburn, Department Microbiology Immunology, Biotechnology Laboratory, The University of British Columbia, 300-6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada.
E-mail: kilburn@unixg.ubc.ca
SOURCE: Biochemical Journal, (15 APR 1999), 339/2 (429-434), 33 reference(s)
CODEN: BIJOAK ISSN: 0264-6021
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1999:29209975 BIOTECHNO
AB A chimaera of stem cell factor (SCF) and a cellulose-binding domain from the xylanase Cex (**CBD**(Cex)) effectively immobilizes SCF on a cellulose surface. The **fusion protein** retains both the cytokine properties of SCF and the cellulose-binding characteristics of **CBD**(Cex). When adsorbed on cellulose, SCF-**CBD**(Cex) is up to 7-fold more potent than soluble SCF-**CBD**(Cex) and than native SCF at stimulating the proliferation of factor-dependent cell lines. When cells are incubated with cellulose-bound SCF-**CBD**(Cex), activated receptors and SCF-**CBD**(Cex) colocalize on the cellulose matrix. The strong binding of SCF-**CBD**(Cex) to the cellulose surface permits the effective and localized stimulation of target cells; this is potentially significant for long-term perfusion culturing of factor-dependent cells. It also permits the direct analysis of the effects of surface-bound cytokines on target cells.

L3 ANSWER 94 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1997:27243859 BIOTECHNO
TITLE: Imobilization of β-glucosidase using the cellulose-binding domain of Bacillus subtilis endo-β-1,4-glucanase
AUTHOR: Ahn D.H.; Kim H.; Pack M.Y.
CORPORATE SOURCE: H. Kim, Department of Agricultural Chemistry, Sunchon National University, Sunchon 540-742, South Korea.
SOURCE: Biotechnology Letters, (1997), 19/5 (483-486), 16 reference(s)
CODEN: BILED3 ISSN: 0141-5492
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1997:27243859 BIOTECHNO
AB A recombinant plasmid p.beta.**CBD** was constructed for immobilization of Cellulomonas fimi β-glucosidase (Cbg) using the cellulose-binding domain (**CBD**) of Bacillus subtilis BSE 616 endo-β-1,4-glucanase (Beg). The Cbg- **CBD**(Beg) **fusion protein**, 80 kDa, was expressed in Escherichia coli and immobilized to Avicel. Cellobiose was completely hydrolyzed with

the immobilized **fusion protein**. The **fusion protein** bound to Avicel retained full activity during continuous operation for 24 h at 4°C.

L3 ANSWER 95 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1997:27065127 BIOTECHNO
TITLE: Comparison of the adsorption properties of a single-chain antibody fragment fused to a fungal or bacterial cellulose-binding domain
AUTHOR: Reinikainen T.; Takkinen K.; Teeri T.T.
CORPORATE SOURCE: Dr. T.T. Teeri, VTT Biotechnology and Food Research, PO Box 1500, FIN-02044 VTT, Finland.
SOURCE: Enzyme and Microbial Technology, (1997), 20/2 (143-149), 44 reference(s)
CODEN: EMTED2 ISSN: 0141-0229
PUBLISHER ITEM IDENT.: S0141022996001093
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1997:27065127 BIOTECHNO
AB Trichoderma reesei cellobiohydrolase I (CBHI) and Cellulomonas fimi cellulase-xylanase (Cex) both have distinct C-terminal cellulose-binding domains which belong to different **CBD** sequence families. Two **fusion proteins** comprising a single-chain antibody fragment (OxscFv) against 2-phenyloxazolone fused to the two **CBDs** (**CBD**(CBHI) or **CBD**(Cex) were constructed. The binding properties of the **fusion proteins** were studied on different cellulosic substrates. It was shown that the **CBD**(Cex) binds the **fusion protein** to cellulose more effectively than the **CBD**(CBHI); however, once immobilized, both **fusion proteins** could be eluted from cellulose only with denaturing agents or very low or high pH. Both **fusion proteins** retained equally well their ability to bind the hapten recognized by their antibody part.

L3 ANSWER 96 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1995:25193346 BIOTECHNO
TITLE: Production and properties of a bifunctional **fusion protein** that mediates attachment of Vero cells to cellulosic matrices
AUTHOR: Wierzba A.; Reichl U.; Turner R.F.B.; Warren R.A.J.; Kilburn D.G.
CORPORATE SOURCE: Dept. of Microbiology/Immunology, University of British Columbia, 237-6174 University Boulevard,Vancouver, BC V6T 1Z3, Canada.
SOURCE: Biotechnology and Bioengineering, (1995), 47/2 (147-154)
CODEN: BIBIAU ISSN: 0006-3592
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1995:25193346 BIOTECHNO
AB The sequence Arg-Gly-Asp (RGD) in extracellular matrix proteins such as fibronectin, collagen, and laminin mediates cell attachment by interacting with proteins of the integrin family of cell surface receptors. A gene fusion encoding the RGD-containing peptide, fused to the C-terminus of a cellulose- binding domain (**CBD**/RGD), was expressed in Escherichia coli. Cultures produced up to 50 mg of **CBD**/RGD per liter, most of which was extracellular. It was

purified from the culture supernatant by affinity chromatography on cellulose. **CBD**/RGD promoted the attachment of green monkey Vero cells to polystyrene and cellulose acetate. Attachment was inhibited by small synthetic peptides containing the RGD sequence, **CBD**/RGD was as effective as collagen in promoting the attachment of Vero cells to Cellsnow(TM) microcarriers.

L3 ANSWER 97 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1994:24178577 BIOTECHNO
TITLE: A streptavidin-cellulose-binding domain **fusion protein** that binds biotinylated proteins to cellulose
AUTHOR: Le K.D.; Gilkes N.R.; Kilburn D.G.; Miller Jr. R.C.; Saddler J.N.; Warren R.A.J.
CORPORATE SOURCE: Department of Microbiology, University of British Columbia, 300-6174 University Boulevard,Vancouver, BC V6T 1Z3, Canada.
SOURCE: Enzyme and Microbial Technology, (1994), 16/6 (496-500)
CODEN: EMTED2 ISSN: 0141-0229
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1994:24178577 BIOTECHNO
AB A **fusion protein**, Sta-**CBD**(Cex), which comprises streptavidin with a cellulose-binding domain (**CBD**(Cex)) fused to its C terminus, was produced in the cytoplasm of Escherichia coli, where it formed inclusion bodies. Renatured Sta-**CBD**(Cex), recovered from the inclusion bodies, adsorbed to Avicel, a microcrystalline cellulose. The cellulose-bound Sta-**CBD**(Cex) in turn bound biotinylated alkaline phosphatase or biotinylated,β-glucosidase. The immobilized β-glucosidase remained fully active during 2 weeks of continuous column operation at 50°C.

L3 ANSWER 98 OF 224 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1993:23293209 BIOTECHNO
TITLE: Production and properties of a factor X-cellulose-binding domain **fusion protein**
AUTHOR: Assouline Z.; Shen H.; Kilburn D.G.; Warren R.A.J.
CORPORATE SOURCE: Department of Microbiology, University of British Columbia, 300-6174 University Boulevard,Vancouver, BC V6T 1Z3, Canada.
SOURCE: Protein Engineering, (1993), 6/7 (787-792)
CODEN: PRENE0 ISSN: 0269-2139
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1993:23293209 BIOTECHNO
AB A **fusion protein**, FX-**CBD**(Cex), which comprises factor X with a cellulose-binding domain (**CBD**(Cex)) fused to its C-terminus, was produced in BHK cells. It was purified from the culture medium by affinity chromatography on cellulose. FX-**CBD**(Cex) could be activated to FXa-**CBD**(Cex) with Russell viper venom. FXa-**CBD**(Cex) was as active as FXa against a chromogenic substrate and against proteins containing the Ile-Glu-Gly-Arg sequence hydrolysed by FXa. FXa-**CBD**(Cex) retained its activity when adsorbed to cellulose.

L3 ANSWER 99 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 8

ACCESSION NUMBER: 2005:515832 BIOSIS
DOCUMENT NUMBER: PREV200510294275
TITLE: Stability analysis of Bacillus stearothermopilus L1 lipase fused with a cellulose-binding domain.
AUTHOR(S): Hwang, Sangpill; Ahn, Ik-Sung [Reprint Author]
CORPORATE SOURCE: Yonsei Univ, Dept Chem Engn, Seoul 120749, South Korea
iahn@yonsei.ar.kr
SOURCE: Biotechnology and Bioprocess Engineering, (JUL-AUG 2005) Vol. 10, No. 4, pp. 329-333.
ISSN: 1226-8372.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Nov 2005
Last Updated on STN: 23 Nov 2005

AB This study was designed to investigate the stability of a lipase fused with a cellulose-binding domain (**CBD**) to cellulase. The **fusion protein** was derived from a gene cluster of a **CBD** fragment of a cellulase gene in Trichoderma hazianum and a lipase gene in Bacillus stearothermophilus L1. Due to the **CBD**, this lipase can be immobilized to a cellulose material. Factors affecting the lipase stability were divided into the reaction-independent factors (RIF), and the reaction-dependent factors (RDF). RIF includes the reaction conditions such as pH and temperature, whereas substrate limitation and product inhibition are examples of RDF. As pH 10 and 50 degrees C were found to be optimum reaction conditions for oil hydrolysis by this lipase, the stability of the free and the immobilized lipase was studied under these conditions. Avicel (microcrystalline cellulose) was used as a support for lipase immobilization. The effects of both RIF and RDF on the enzyme activity were less for the immobilized lipase than for the free lipase. Due to the irreversible binding of **CBD** to Avicel and the high stability of the immobilized lipase, the enzyme activity after five times of use was over 70% of the initial activity.

L3 ANSWER 100 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 11

ACCESSION NUMBER: 2005:191597 BIOSIS
DOCUMENT NUMBER: PREV200500193288
TITLE: Construction of recombinant Escherichia coli for over-production of soluble heparinase I by fusion to maltose-binding protein.
AUTHOR(S): Chen, Yin; Xing, Xin-Hui [Reprint Author]; Lou, Kai
CORPORATE SOURCE: Dept Chem Engn, Tsing Hua Univ, Beijing, 100084, China
xhxing@tsinghua.edu.cn
SOURCE: Biochemical Engineering Journal, (April 2005) Vol. 23, No. 2, pp. 155-159. print.
ISSN: 1369-703X (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 May 2005
Last Updated on STN: 25 May 2005

AB Heparinase I (EC 4.2.2.7) is one of the three heparinases purified from Flavobacterium heparinum that cleaves certain sequences of heparin/heparan sulfate specifically. Previous reports have shown that this enzyme expressed in recombinant Escherichia coli was highly prone to aggregate into inactive inclusion bodies even by fusion to cellulose-binding domain (**CBD**). In this paper, we fused heparinase I to maltose-binding protein (MBP) and expressed the **fusion protein** in E. coli to develop an expression system of soluble heparinase I. As a result, about 90% of the **fusion protein** (abbreviated as MBP-hepA) was soluble when expressed in the recombinant E. coli and the

fusion protein could reach about 100 mg l-1 with an activity of 88.3 Ul-1 OD600-1. To our knowledge, this is the first time to produce soluble heparinase I at such a high yield. Copyright 2004 Elsevier B.V. All rights reserved.

L3 ANSWER 101 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 36

ACCESSION NUMBER: 2002:371642 BIOSIS
DOCUMENT NUMBER: PREV200200371642
TITLE: Modeling of growth and energy metabolism of Pichia pastoris producing a **fusion protein**.
AUTHOR(S): Jahic, M.; Rotticci-Mulder, J. C.; Martinelle, M.; Hult, K.; Enfors, S.-O. [Reprint author]
CORPORATE SOURCE: Department of Biotechnology, Royal Institute of Technology, Roslagstullbacken 21, 10691, Stockholm, Sweden olle@biochem.kth.se
SOURCE: Bioprocess and Biosystems Engineering, (March, 2002) Vol. 24, No. 6, pp. 385-393. print.
ISSN: 1615-7591.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Jul 2002
Last Updated on STN: 3 Jul 2002

AB A **fusion protein** composed of a cellulose binding domain from Neocallimastix patriciarum cellulase A and Candida antarctica lipase B (**CBD**-lipase) was produced by Pichia pastoris methanol utilization plus phenotype in high cell-density cultures. The genes expressing **CBD**-lipase were fused to the alpha-factor secretion signal sequence of Saccharomyces cerevisiae and placed under the control of the alcohol oxidase gene (AOX1) promoter. To control the repression and induction of AOX1 and oxygen demand at high cell density, a four-stage process was used. Batch growth on glycerol was used in the first step to provide biomass (28 g L-1) while product formation was prevented due to repression of the AOX1. The second stage was exponential fed-batch growth on glycerol, which caused a slight increase of the enzyme alcohol oxidase activity due to derepression of the AOX1. This procedure resulted in smooth transition to exponential fed-batch growth on methanol, the third stage, in which the AOX1 was strongly induced. The fourth stage was constant fed-batch growth on methanol used to control the oxygen demand at the high cell density. A kinetic model was developed that could predict biomass growth and oxygen consumption in processes with and without oxygen-enriched air. With oxygen enrichment to 34% O2 in the inlet air the methanol feed rate could be increased by 50% and this resulted in 14% higher final cell density (from 140 to 160 g L-1 cell dry weight). The increased methanol feed rate resulted in a proportionally increased specific rate of product secretion to the medium. After an initial decrease, the synthesis capacity of the cell was kept constant throughout the cultivation, which made the product concentration increase almost constantly during the process. The kinetic model also describes how the low maintenance demand of P. pastoris compared with E. coli enables this organism to grow to such high cell densities.

L3 ANSWER 102 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 48

ACCESSION NUMBER: 2001:277343 BIOSIS
DOCUMENT NUMBER: PREV200100277343
TITLE: Non-hydrolytic disruption of crystalline structure of cellulose by cellulose binding domain and linker sequence of cellobiohydrolase I from Penicillium janthinellum.
AUTHOR(S): Gao Pei-Ji [Reprint author]; Chen Guan-Jun; Wang Tian-Hong; Zhang Ying-Shu; Liu-Jie
CORPORATE SOURCE: State key Laboratory of Microbial Technology, Shandong University, Jinan, 250100, China

gaopj@sdu.edu.cn
SOURCE: Shengwu Huaxue yu Shengwu Wuli Xuebao, (2001) Vol. 33, No. 1, pp. 13-18. print.
ISSN: 0582-9879.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Jun 2001
Last Updated on STN: 19 Feb 2002

AB The cooperation between cellobiohydrolase (CBHI) and endoglucanase (EG) is necessary for biodegradation of native cellulose, but its mechanism is still poorly understood. The present paper report at the first time that an isolated component, the cellulose binding domain with its linker sequence of cellobiohydrolase I from Penicillium janthinellum (CBDCBHI), plays an important role in the synergism between CBHI and EGI during cellulose biodegradation. A recombinant plasmid (pUC18C), containing the gene fragment encoding CBDCBHI from P. janthinellum was derived from pUC18-181. In pUC 18C, the catalytic domain region of cbh I gene was deleted by in vitro DNA manipulations and then E. coli JM 109 was transformed for the production of LacZ-**CBD fusion protein**. The active LacZ-**CBD fusion protein** was digested by papain and then purified by re-exclusion chromatography. The purified peptide sequence of CBDCBHI had the ability of binding crystalline cellulose. The detailed morphological and structural changes of cotton fibers after binding CBDCBHI were investigated by using scanning electron microscopy, calorimetric activity and X-ray diffraction. The results demonstrated that the CBDCBHI not only has a high binding capacity to cellulose, but also causes non-hydrolytic disruption of crystalline cellulose, which leads to the release of short fibers. IR spectroscopy and X-ray diffraction show that destabilization is caused by the non-hydrolytic disruption of cellulose and the disruption of hydrogen bonds in crystalline cellulose. The efficiency of crystalline cellulose degradation was enhanced by synergistic action of CBDCBHI with EGI. These results suggest that the cellulose-binding domain with its linker plays an important role in crystalline cellulose degradation.

L3 ANSWER 103 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 70
ACCESSION NUMBER: 1997:420979 BIOSIS
DOCUMENT NUMBER: PREV199799720182
TITLE: Cellulose binding domain expression vectors for the rapid, low cost purification of **CBD-fusion proteins**.
AUTHOR(S): Novy, R. [Reprint author]; Yaeger, K. [Reprint author]; Monsma, S. [Reprint author]; McCormick, M. [Reprint author]; Berg, J. [Reprint author]; Shoseyov, O.; Shpigel, E.; Seigel, D.; Goldlust, A.; Efroni, G.; Singer, Y.; Kilburn, D.; Tomme, P.; Gilkes, N.
CORPORATE SOURCE: Novagen Inc., Madison, WI, USA
SOURCE: FASEB Journal, (1997) Vol. 11, No. 9, pp. A1151.
Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology. San Francisco, California, USA. August 24-29, 1997.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Oct 1997
Last Updated on STN: 8 Oct 1997

L3 ANSWER 104 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:544204 BIOSIS
DOCUMENT NUMBER: PREV200300545978
TITLE: Production of recombinant chinchilla beta-defensin 1 in Escherichia coli.
AUTHOR(S): Harris, R. H. [Reprint Author]; Rawale, S. V.; Kaumaya, P. T. P.; Munson, R. S. Jr. [Reprint Author]; Bakaletz, L. O. [Reprint Author]
CORPORATE SOURCE: Coll. of Medicine and Public Health, Columbus Children's Research Institute, Ohio State Univ., Columbus, OH, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. E-012. http://www.asmusa.org/mtgsrc/generalmeeting.htm. cd-rom. Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003. American Society for Microbiology. ISSN: 1060-2011 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 19 Nov 2003
Last Updated on STN: 19 Nov 2003

AB Otitis media is caused by opportunistic pathogens that ordinarily colonize the healthy pediatric nasopharynx without incident. During colonization, these pathogens are exposed to effectors of the innate immune system that likely contribute to maintaining bacterial numbers at a non-infectious level. beta-defensins are a key element of this innate immune surveillance system as they are a class of antimicrobial peptides produced by the mammalian mucosa whose role is to protect against invading microbes. Previously, we described the identification of a beta-defensin expressed in the upper airway including the nasopharynx, Eustachian tube, and trachea of the chinchilla, the primary host for studying the viral and bacterial pathogens of otitis media. In order to perform a detailed characterization of this defensin, we have expressed chinchilla beta-defensin 1(**cBD**-1) as a His-tagged **fusion protein** in Escherichia coli. The DNA encoding the mature 45 amino acid peptide was cloned into pET30a under the control of the T7 phage RNA polymerase promoter. Expression of **cBD**-1 was induced with IPTG in E. coli strain BL21 (DE3), and the recombinant peptide was recovered from the insoluble portion of the cell sonicate via the His tag. After cleavage and removal of the His tag, recombinant **cBD**-1 was found to be greater than 80% homogeneous by RP-HPLC. MALDI-TOF MS indicated that the mass of the peptide was 5124 Da which is consistent with the calculated mass of 5124.16 Da. The C-terminal fragment of **cBD**-1 constitutes the putative antimicrobial portion of the peptide based on its 77% amino acid identity with human beta-defensin 3, whose active form is known. As human beta-defensin 3 is active against various microorganisms at sub-micromolar concentrations, we expect recombinant **cBD**-1 to have a similar spectrum of activity.

L3 ANSWER 105 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:498264 BIOSIS
DOCUMENT NUMBER: PREV200200498264
TITLE: Characterization and expression in-planta of a fungal cellulose-binding domain.
AUTHOR(S): Quentin, Michael [Reprint author]; Derksen, Jan; DeJong, Ed [Reprint author]; Mariani, Celestina; VanderValk, Henry [Reprint author]
CORPORATE SOURCE: Department of Fibre and Paper Technology, ATO BV, 6700 AA, P.O. Box 17, Wageningen, Netherlands m.g.e.quentin@ato.wag-ur.nl; e.dejong@ato.wag-ur.nl
SOURCE: Abstracts of Papers American Chemical Society, (2002) Vol. 223, No. 1-2, pp. CELL 99. print.

Meeting Info.: 223rd National Meeting of the American Chemical Society. Orlando, FL, USA. April 07-11, 2002.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Sep 2002
Last Updated on STN: 25 Sep 2002

L3 ANSWER 106 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:290779 BIOSIS
DOCUMENT NUMBER: PREV200100290779
TITLE: **CBD**-Factor X **fusion protein** production by a stable transformed Sf9 insect cell line in a high cell density perfusion culture.
AUTHOR(S): Gorenflo, Volker M. [Reprint author]; Pfeifer, Tom A.; Grigliatti, Thomas A.; Lesnicki, Gary; Kilburn, Doug G.; Piret, James M. [Reprint author]
CORPORATE SOURCE: Biotechnology Laboratory and Department of Chemical and Biological Engineering, University of British Columbia, 6174 University Boulevard, Vancouver, BC, V6T 1Z3, Canada volker@interchange.ubc.ca
SOURCE: Abstracts of Papers American Chemical Society, (2001) Vol. 221, No. 1-2, pp. BIOT 147. print.
Meeting Info.: 221st National Meeting of the American Chemical Society. San Diego, California, USA. April 01-05, 2001. American Chemical Society.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Jun 2001
Last Updated on STN: 19 Feb 2002

L3 ANSWER 107 OF 224 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:177814 BIOSIS
DOCUMENT NUMBER: PREV200000177814
TITLE: Recombinant protein purification using **CBDS**.
AUTHOR(S): Shpigel, E. [Reprint author]; Goldlust, A. [Reprint author]; Eshel, A. [Reprint author]; Shoseyov, O.; Kilburn, D. G.; Gilkes, N.; Guarna, M. M.; Kwan, E. M.; Boraston, A. B.; Warren, R. A. J.
CORPORATE SOURCE: CBD-Technologies Ltd, Park Tamar, Rehovot, 76100, Israel
SOURCE: Abstracts of Papers American Chemical Society, (2000) Vol. 219, No. 1-2, pp. BIOT 20. print.
Meeting Info.: 219th Meeting of the American Chemical Society. San Francisco, California, USA. March 26-30, 2000. American Chemical Society.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 11 May 2000
Last Updated on STN: 4 Jan 2002

L3 ANSWER 108 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2006:149298 CAPLUS
DOCUMENT NUMBER: 144:211227
TITLE: Methods and compositions for concentrating secreted recombinant protein
INVENTOR(S): Taron, Christopher H.; Colussi, Paul A.

PATENT ASSIGNEE(S): New England Biolabs, Inc., USA
SOURCE: U.S. Pat. Appl. Publ., 32 pp., Cont.-in-part of U.S. Ser. No. 110,002.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2006035333	A1	20060216	US 2005-242553	20051003
US 2003216550	A1	20031120	US 2003-375913	20030226
US 6897285	B2	20050524		
US 2005227326	A1	20051013	US 2005-102475	20050408
US 2005196804	A1	20050908	US 2005-110001	20050420
US 6984505	B2	20060110		
US 2005196841	A1	20050908	US 2005-110002	20050420
US 6987007	B2	20060117		
US 2006030008	A1	20060209	US 2005-235009	20050926
WO 2006041849	A2	20060420	WO 2005-US35697	20051003

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRIORITY APPLN. INFO.:

US 2002-360354P	P	20020228
US 2003-375913	A3	20030226
US 2004-560418P	P	20040408
US 2004-616420P	P	20041006
US 2005-102475	A2	20050408
US 2005-110001	A2	20050420
US 2005-110002	A2	20050420
US 2005-690470P	P	20050614

AB Methods and compns. are described that relate to obtaining concentrated prepns. of secreted recombinant proteins. These proteins are expressed in the form of **fusion proteins** with a chitin-binding domain (**CBD**). The **fusion proteins** are capable of being concentrated in the presence of chitin. Also described is: a shuttle vector that includes a modified LAC4 promoter; a chitinase-neg. host cell; a **CBD** capable of eluting from chitin under non-denaturing conditions; and sterilized chitin, which can be optionally magnetized for facilitating recovery of recombinant protein.

L3 ANSWER 109 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2006:169085 CAPLUS
DOCUMENT NUMBER: 144:365669
TITLE: Adsorption of pure recombinant MBP-**fusion proteins** on amylose affinity membranes
AUTHOR(S): Cattoli, Francesca; Boi, Cristiana; Sorci, Mirco; Sarti, Giulio C.
CORPORATE SOURCE: Dipartimento di Ingegneria Chimica, Mineraria e Delle Tecnologie Ambientali, Universita di Bologna, Bologna, 40136, Italy
SOURCE: Journal of Membrane Science (2006), 273(1-2), 2-11
CODEN: JMESDO; ISSN: 0376-7388

PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The specific interaction between MBP-**fusion proteins** and amylose based affinity membranes has been presented recently. In this work, the attention is focused on the influence of ionic strength on the equilibrium isotherm and on the kinetics of adsorption and desorption. Three different MBP-**fusion proteins** have been used: MBP-rubredoxin (MW 51 kDa), MBP-intein-**CBD** (MW 97 kDa) and MBP-β galactosidase (MW 160 kDa) characterized by different dimensions. The equilibrium data follow the Langmuir isotherm, whose parameters qm and Kd show a dependence on salt concentration in the feed solution
By increasing the ionic strength, the binding capacity at saturation qm decreases while the equilibrium constant Kd increases, indicating that a higher concentration of NaCl reduces the affinity interaction between protein and ligand. A simple kinetic model has been investigated for the interpolation of adsorption and elution curves. No mass transfer effects have been considered within the liquid phase and inside the pores of the stationary phase. The high processing speed of membrane affinity filtration has been exploited. The kinetics of adsorption and elution have been described and the values of the kinetic consts. have been obtained both for adsorption and elution conditions for all proteins. The influence of the action of a competing substrate like maltose on the removal of the immobilized product has been also determined
REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 110 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 13
ACCESSION NUMBER: 2004:718682 CAPLUS
DOCUMENT NUMBER: 141:237743
TITLE: Use of caspase for recovery processing of engineered recombinant **fusion proteins**
INVENTOR(S): Mertens, Nico Maurice August Corneel; Kelly, Andrew Graham
PATENT ASSIGNEE(S): Biotecnol S.A., Port.; Vlaams Interuniversitair Instituut voor Biotechnologie; Universiteit Gent
SOURCE: PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004074488	A1	20040902	WO 2004-EP1759	20040223
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2004213586	A1	20040902	AU 2004-213586	20040223
CA 2511855	AA	20040902	CA 2004-2511855	20040223
EP 1597369	A1	20051123	EP 2004-713540	20040223
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
NO 2005003033	A	20050620	NO 2005-3033	20050620
US 2006099689	A1	20060511	US 2005-543587	20050902
PRIORITY APPLN. INFO.:			EP 2003-447034 A	20030221

WO 2004-EP1759 W 20040223

AB This invention describes the use of caspases as maturation proteases for recombinant **fusion proteins** containing an engineered caspase recognition site in the linker connecting the **fusion protein** and the protein of interest. The present invention describes a process using caspases to sep. the fusion part and the linker sequence from a protein of interest without limitation of choice of the first amino acid of the mature protein, making the method suitable for producing human and animal therapeutics. The invention also relates to the design of peptide linkers used to connect a fusion partner to the mature protein whereby said linker is specifically digested by such a caspase. The invention further relates to the expression and purification of **fusion proteins** comprising a fusion part and a mature protein or peptide of interest comprising a linker sequence designed to be processed by a caspase protease. This invention can be used to facilitate production of a recombinant protein in a functional and/or mature form and to facilitate recovery processes. The present invention results in a method that cleaves the fusion part and the linker sequence with high specificity, and several specificities can be engineered in the linker depending on the caspase used. Surprisingly, it was found that even proteins with suspected cleavage sites on the basis of their protein sequence were only cut at the engineered site and not at cryptic internal sites. Furthermore, cleavage times can be as short as several minutes to one hour. The authors conclude that the caspases are a good choice to be used as a processing enzyme for **fusion proteins** since any amino acid can be tolerated at the P1' position, they are efficient in cutting (usually complete after 45 min reaction time), and the sequence specificity is sufficient to prefer the processing at the engineered site, even if the target protein contains consensus recognition sequences for the caspase enzymes.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 111 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 21

ACCESSION NUMBER: 2003:805688 CAPLUS
DOCUMENT NUMBER: 139:318415
TITLE: Preparation of human fusion N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase
INVENTOR(S): Habuchi, Osami; Otake, Shiori; Ito, Yuki; Fukuda, Masakazu; Muramatsu, Takashi; Muramatsu, Sumiko; Ichihara, Keiko; Zhou, Pang
PATENT ASSIGNEE(S): Seikagaku Kogyo Co., Ltd., Japan; Kazusa DNA Research Institute Foundation
SOURCE: Jpn. Kokai Tokkyo Koho, 24 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2003289882	A2	20031014	JP 2002-272334	20020918
PRIORITY APPLN. INFO.:			JP 2002-24874 A	20020131

AB This invention provides method of preparation of human **fusion protein** comprising N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase (GlcNac4S-6ST) and expression tag. The proteins sequences of human GlcNac4S-6ST was disclosed. The N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase catalyzed transfer of sulfate position 6 of chondroitin sulfate. The method provided in this method can be used for mass production of N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase.

L3 ANSWER 112 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 22

ACCESSION NUMBER: 2003:989729 CAPLUS
DOCUMENT NUMBER: 140:37062
TITLE: Use of **fusion protein** with cell surface exposed cellulose binding domain to improve cellular adhesion of bacteria to plant roots
INVENTOR(S): Vande, Broek Ann; Van Boxel, Nadja; Vanderleyden, Jozef; Goddard, Peter; Srinivasan, Murali
PATENT ASSIGNEE(S): Norsk Hydro As, Norway
SOURCE: Brit. UK Pat. Appl., 92 pp.
CODEN: BAXXDU
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
GB 2389584	A1	20031217	GB 2002-13760	20020614
WO 2003106490	A1	20031224	WO 2003-GB2520	20030611
WO 2003106490	B1	20040219		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003240089	A1	20031231	AU 2003-240089	20030611

PRIORITY APPLN. INFO.:

GB 2002-13760	A	20020614
WO 2003-GB2520	W	20030611

AB The present invention provides a **fusion protein** comprising: a cellulose binding domain (**CBD**) peptide; and a cell surface polypeptide capable of causing the cellulose binding domain peptide to be exposed on the surface of a cell. There is provided the use of a cellulose binding domain peptide to improve cellular adhesion. According to another aspect of the present invention, there is provided a method of improving plant growth or health comprising applying a cell as described above to the roots of a plant. The present invention has arisen from the finding that the presence of cellulose binding domains on the surface of bacteria improves the binding of the bacteria to plant roots. The DNA encoding the cellulose binding domain of cellobiohydrolase I (CBHI) in Trichoderma reesei was inserted into the gene for the major outer membrane protein (omaA or momP gene) of Azospirillum brasilense, flagellin protein of the lateral flagella (lafl gene) of Azospirillum brasilense, and the major outer membrane protein (OprF gene) of P. fluorescens, to generate a construct encoding a **fusion protein**. These exptl. results also showed that the **CBD** peptide is present on the cell surface of the transformed cells.
REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 113 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 26
ACCESSION NUMBER: 2003:830939 CAPLUS
DOCUMENT NUMBER: 140:88376
TITLE: **Fusion proteins** containing Coprinus cinereus peroxidase and the cellulose-binding domain of Humicola insolens family 45 endoglucanase
AUTHOR(S): Xu, Feng; Jones, Aubrey; Lamsa, Michael H.; Fuglsang, Claus C.; Conrad, Lars S.; Kierulff, Jesper V.; Brown, Stephen H.; Cherry, Joel R.

CORPORATE SOURCE: Novozymes Biotech Inc., Davis, CA, 95616, USA
SOURCE: ACS Symposium Series (2003), 855(Applications of Enzymes to Lignocellulosics), 382-402
CODEN: ACSMC8; ISSN: 0097-6156
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two **fusion proteins** were constructed to attach the cellulose-binding domain of Humicola insolens family 45 endo-1,4-glucanase, via the linker of the endoglucanase, to Coprinus cinereus heme peroxidase. One **fusion protein** utilized the wild type peroxidase, and another utilized a peroxidase mutant resistant to alkaline pH, high temperature, and H2O2. The **fusion proteins** were expressed in Aspergillus oryzae and characterized. Their peroxidase activity was similar to that of the donor peroxidase, but their cellulose-binding affinity was 100-fold less than the donor endoglucanase. Neutralizing the surface neg. charge enhanced their affinity to cotton fabric. Although the **fusion proteins** could bind to cellulose, they failed to oxidize cellulose-adsorbed dyes that were their substrates in dissolved state.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 114 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 29
ACCESSION NUMBER: 2003:830925 CAPLUS
DOCUMENT NUMBER: 140:58464
TITLE: Modulation of wood fibers and paper by cellulose-binding domains
AUTHOR(S): Shoseyov, Oded; Levy, Ilan; Shani, Ziv; Mansfield, Shawn D.
CORPORATE SOURCE: The Institute of Plant Sciences and Genetics in Agriculture, the Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, 76100, Israel
SOURCE: ACS Symposium Series (2003), 855(Applications of Enzymes to Lignocellulosics), 116-131
CODEN: ACSMC8; ISSN: 0097-6156
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with refs. Recombinant cellulose-binding domains (**CBD**) have previously been shown to modulate the elongation of different plant cells in vitro. Using Acetobacter xylinum as a model system, **CBD** was found to increase the activity of the cellulose synthase, up to fivefold, in a dose-dependent manner. In Populus, the introduction of a **cbd** gene under the control of the elongation specific cel1 promoter led to significant increases in biomass production in selected clones compared with wild-type plants. An anal. of the ensuing wood characteristics from the transgenic trees demonstrated significant increases in both fiber cell length and the average degree of cellulose polymerization Addnl., a significant decrease in microfibril angle was observed
These results coincided with increased burst, tear and tensile indexes of paper prepared from these wood fibers. The mechanism by which **CBD** affects cell wall metabolism remains unknown. A physio-mech. mechanism was postulated whereby **CBD**. Localizes between adjacent cellulose microfibrils and separates them in a wedge-like action. In vitro expts. with petunia cell suspensions supports this hypothesis, as increasing concns. of **CBD** displayed an abnormal shedding of cell wall layers, indicating that **CBD** has the potential to cause non-hydrolytic cell wall disruption activity in-vivo. Addnl., **CBD** **fusion proteins** may also be used to cross-link and introduce functional mols. into and onto lignocellulosic-based fiber

networks. Consequently, these **CBD** fused mols. can be used to improve the physio-mech. performance of paper sheets, and to alter its surface properties.

REFERENCE COUNT: 67 THERE ARE 67 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 115 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 55

ACCESSION NUMBER: 1999:34437 CAPLUS
DOCUMENT NUMBER: 130:107243
TITLE: Methods of detection using a cellulose binding domain fusion product
INVENTOR(S): Shoseyov, Oded; Shpiegl, Itai; Goldstein, Marc A.; Doi, Roy H.
PATENT ASSIGNEE(S): Yissum Research Development Company of the Hebrew University of Jerusalem, Israel; The University of California
SOURCE: U.S., 63 pp., Cont.-in-part of U.S. 5,496,934.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5856201	A	19990105	US 1994-330394	19941027
US 5496934	A	19960305	US 1993-48164	19930414
CA 2160670	AA	19941027	CA 1994-2160670	19940414
CN 1125452	A	19960626	CN 1994-192440	19940414
CN 1059214	B	20001206		
US 5670623	A	19970923	US 1995-460462	19950602
US 5719044	A	19980217	US 1995-460457	19950602
US 5738984	A	19980414	US 1995-460458	19950602
US 5837814	A	19981117	US 1995-460455	19950602
CN 1217339	A	19990526	CN 1998-118445	19980813
CN 1223377	A	19990721	CN 1998-118443	19980813
PRIORITY APPLN. INFO.:			US 1993-48164 A2	19930414

AB A cellulose binding domain (**CBD**) having a high affinity for crystalline cellulose and chitin is disclosed, along with methods for the mol. cloning and recombinant production thereof. Fusion products comprising the **CBD** and a second protein (binding to target substances) are likewise described. The fusion products are useful in detecting target substances. IgG was purified from human serum using recombinantly-prepared **CBD**-protein A fusion product bound to cellulose.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 116 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 56

ACCESSION NUMBER: 1999:530317 CAPLUS
DOCUMENT NUMBER: 131:181955
TITLE: Purification of recombinantly prepared proteins by using the cellulose-binding domain of a cellulose-degrading enzyme as an affinity tag
INVENTOR(S): Karita, Shuichi; Ohmiya, Kunio; Sakka, Kazuo; Kimura, Tetsuya
PATENT ASSIGNEE(S): Toyobo Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 11225763	A2	19990824	JP 1998-29410	19980212

PRIORITY APPLN. INFO.: JP 1998-29410 19980212

AB Purification of a protein (enzyme, antibody, or hormone) that is expressed as a **fusion protein** with the cellulose-binding domain (**CBD**) of a cellulose-degrading enzyme is described. The **fusion protein**-containing cellular extract is first mixed with an insol., non-crystal cellulose carrier for absorption; the absorbed **fusion protein** is then eluted with a carbohydrate such as cellobiose, maltose, glucose, or xylose. The target protein is then retrieved from the purifd. **fusion protein** by digestion with a proteinase such as trypsin. Purification of endoglucanase IV of Ruminococcus albus fused with the **CBD** of xylanase A of Clostridium stercorarium was described.

L3 ANSWER 117 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 63

ACCESSION NUMBER: 1998:251260 CAPLUS

DOCUMENT NUMBER: 128:318808

TITLE: **Fusion protein** comprising α-amylase and a cellulose-binding domain for the degradation of starch

INVENTOR(S): Bjornvad, Mads; Pedersen, Sven; Schulein, Martin; Bisgard-Frantzen, Henrik

PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.; Bjornvad, Mads; Pedersen, Sven; Schulein, Martin; Bisgard-Frantzen, Henrik

SOURCE: PCT Int. Appl., 84 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9816633	A1	19980423	WO 1997-DK448	19971013
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
EP 950093	A2	19991020	EP 1997-943797	19971013
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
CN 1233286	A	19991027	CN 1997-198640	19971013

PRIORITY APPLN. INFO.: DK 1996-1130 A 19961011
WO 1997-DK448 W 19971013

AB The invention relates to a starch conversion method wherein the starch substrate is treated in aqueous medium with a cellulose-binding domain (**CBD**)/enzyme hybrid. Further, the invention also relates to an isolated DNA sequence encoding a stable **CBD**/enzyme hybrid, a DNA construct comprising said DNA sequence of the invention, an expression vector comprising the DNA sequence of the invention, and a **CBD** /enzyme hybrid. One such hybrid comprises Termamyl (a Bacillus α-amylase) fused by the linker peptide SDPDSGEPDPTPPSDPG to the most C-terminal **CBD** (residues 417-462) of alkaline cellulase C315A from Bacillus agaradherens NCIMB 40482. Similarly, Termamyl is linked via RPPTPTSPSAPS to the Humicola insolens endoglucanase V **CBD**. The hybrid enzymes give improved starch liquefaction per μg enzyme per g DS when compared to Termamyl alone.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 118 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 80
ACCESSION NUMBER: 1995:372817 CAPLUS
DOCUMENT NUMBER: 122:181411
TITLE: A novel cellulose binding domain with high affinity for cellulose and chitin and its applications
INVENTOR(S): Shoseyov, Oded; Shpiegl, Itai; Goldstein, Marc A.; Doi, Roy H.
PATENT ASSIGNEE(S): Regents of the University of California, USA; Yissum Research Development Co.
SOURCE: PCT Int. Appl., 125 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9424158	A1	19941027	WO 1994-US4132	19940414
W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, UA, US, UZ				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5496934	A	19960305	US 1993-48164	19930414
CA 2160670	AA	19941027	CA 1994-2160670	19940414
AU 9466347	A1	19941108	AU 1994-66347	19940414
AU 691807	B2	19980528		
EP 695311	A1	19960207	EP 1994-914175	19940414
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
CN 1125452	A	19960626	CN 1994-192440	19940414
CN 1059214	B	20001206		
JP 08509127	T2	19961001	JP 1994-523460	19940414
US 5670623	A	19970923	US 1995-460462	19950602
US 5719044	A	19980217	US 1995-460457	19950602
US 5738984	A	19980414	US 1995-460458	19950602
US 5837814	A	19981117	US 1995-460455	19950602
NO 9504074	A	19951127	NO 1995-4074	19951013
FI 9504888	A	19951213	FI 1995-4888	19951013
CN 1217339	A	19990526	CN 1998-118445	19980813
CN 1223377	A	19990721	CN 1998-118443	19980813
PRIORITY APPLN. INFO.:			US 1993-48164 A	19930414
			WO 1994-US4132 W	19940414

AB A novel cellulose binding domain (**CBD**) with a high affinity for crystalline cellulose and chitin is described and a DNA sequence encoding it is cloned and expressed. Fusion products of the **CBD** and a second protein are described and have a wide range of applications including drug delivery, affinity sepns., and diagnostic techniques. The **CBD** in this case is derived from cellulose-binding protein A of Clostridium cellulovorans. The coding sequence for the carbohydrate-binding domain was cloned by PCR and expressed in Escherichia coli. Characterization of the **CBD** is described. A chimeric gene for a **fusion protein** of the **CBD** and Protein A was constructed and expressed in Escherichia coli. The **fusion protein** was purified in a single step affinity chromatog. against cellulose. This complex could bind IgG and the IgG could be eluted from the complex by acetic acid 1 M without elution of the **fusion protein**.

L3 ANSWER 119 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:244768 CAPLUS
TITLE: Expression and characterization of the β isoform of glucokinase regulatory protein (GKRP) from Homo

sapiens β-islet cells
AUTHOR(S): Carr, Kevin Ricardo; Taylor, Brandon Christopher; Shipman, Lance Winston
CORPORATE SOURCE: Department of Chemistry, Morehouse College, Atlanta, GA, 30314, USA
SOURCE: Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 (2006), CHED-699. American Chemical Society: Washington, D. C.
CODEN: 69HYEC
DOCUMENT TYPE: Conference; Meeting Abstract; (computer optical disk)
LANGUAGE: English
AB Glucokinase (GK) phosphorylates glucose to glucose-6-phosphate and is modulated by (GKRP), with association of the complex occurring in fructose-6-phosphate (F6P) and dissociation in the presence of fructose-1-phosphate (F1P). Using complementary single-stranded synthetic oligonucleotides to construct duplex DNA corresponding to the 3'-terminus of β-islet mRNA variant, the gene was subcloned and overexpressed in E. coli yielding the β1-GKRP isoform produced in pancreatic β-islet cells described by Alvarez, et. al. After purification by metal chelation affinity chromatog., the variant protein was tested for binding and inhibition of human liver glucokinase. A binding assay was developed using a chitin binding domain/glucokinase (**CBD**/GK) **fusion protein** immobilized on a chitin column against β1-GKRP as the mobile phase. The inhibition of GK by β1-GKRP in the presence of either F6P or F1P was observed and compared to rat liver GKRP by monitoring glucokinase activity coupled to either pyruvate kinase/ lactate dehydrogenase or glucose-6-phosphate dehydrogenase activity.

L3 ANSWER 120 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:93942 CAPLUS
DOCUMENT NUMBER: 144:260666
TITLE: Ultrasound preexposure improves endothelial cell binding and retention on biomaterial surfaces
AUTHOR(S): Hsu, Shan-hui; Huang, Tsung-bin; Chuang, Shang-chi; Tsai, I-Jine; Chen, David C.
CORPORATE SOURCE: Department of Chemical Engineering, National Chung Hsing University, Taichung, Taiwan
SOURCE: Journal of Biomedical Materials Research, Part B: Applied Biomaterials (2006), 76B(1), 85-92
CODEN: JBMRGL; ISSN: 1552-4973
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In spite of the extensive studies regarding the effects of ultrasound on biol. systems, the influence of low-intensity ultrasound on endothelial cells has rarely been investigated. In this work, the effect of ultrasound in improving the binding between endothelial cells and biomaterial substrates was evaluated. Based on the results, low-intensity ultrasound could change the morphol. and matrix secretion of endothelial cells, and such effects persisted when preexposed cells were seeded to another substrate. The cells preexposed to ultrasound were spread further on the substrate. The actin stress fibers of ultrasound-preexposed cells on RGD-modified surfaces were especially intense and well oriented. Ultrasound could probably activate cellular integrins and subsequently allow RGD to bind them. A much firmer adhesion of ultrasound-preexposed endothelial cells to the biomaterial surface coated with the RGD-containing protein was demonstrated. Finally, polyurethane small-diameter vascular grafts seeded with ultrasound-preexposed endothelial cells showed enhanced cell retention on graft surfaces upon flushing.
REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 121 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:696937 CAPLUS
DOCUMENT NUMBER: 143:192300
TITLE: Expression systems comprising heterokaryon fungus host cell and nucleic acids encoding heterologous signal peptide and cellulose-binding domain for industrial scale production of human antibodies
INVENTOR(S): Lehmbeck, Jan; Wahlbom, Fredrik
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005070962	A1	20050804	WO 2005-DK35	20050120
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2006024782	A1	20060202	US 2005-41095	20050120

PRIORITY APPLN. INFO.:		
DK 2004-77	A	20040121
DK 2004-174	A	20040205
DK 2004-761	A	20040512
US 2004-543227P	P	20040209
US 2004-580150P	P	20040616

AB The present invention relates to methods for producing a monoclonal antibody in a heterokaryon fungus or in a fungal host cell. Furthermore, it also relates to a nucleic acid construct a first nucleic acid sequence encoding a light chain of an antibody and a third nucleic acid sequence encoding a signal peptide heterologous to the first nucleic acid sequence, a nucleic acid construct comprising a first nucleic acid sequence encoding a heavy chain of an antibody and a third nucleic acid sequence encoding a signal peptide heterologous to the first nucleic acid sequence, a nucleic acid construct comprising a first nucleic acid sequence encoding a light chain of an antibody and a second nucleic acid sequence encoding a cellulose binding domain, and a nucleic acid construct comprising a first nucleic acid sequence encoding a heavy chain of an antibody and a second nucleic acid sequence encoding a cellulose binding domain. The monoclonal antibodies are human IgG1 or IgG2 heavy and light chains. The heterologous signal peptide is derived from Aspergillus oryzae α-amylase gene, Candida antarctica lipase gene, or Candida antarctica lipase B gene. The cellulose-binding domain is derived from endoglucanase II of Meripilus giganteus. The expression host constructed were hemA- Aspergillus oryzae and pyrG- Aspergillus oryzae. The expression systems of the invention are suitable for industrial scale production
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 122 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:58417 CAPLUS
DOCUMENT NUMBER: 142:110126

TITLE: Specific detection of chitin using chitin-binding domain
INVENTOR(S): Zhang, Yinhua; McReynolds, Larry
PATENT ASSIGNEE(S): New England Biolabs, Inc., Peop. Rep. China; Carlow, Clothilde; Foster, Jeremy; Kumar, Sanjay
SOURCE: PCT Int. Appl., 36 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005005955	A2	20050120	WO 2004-US17979	20040607
WO 2005005955	C1	20050317		
WO 2005005955	A3	20050519		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-483279P P 20030627

AB Methods and kits are provided for detecting chitin in biol. samples using chitin-binding domain (**CBD**).

L3 ANSWER 123 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:138327 CAPLUS
DOCUMENT NUMBER: 144:186016
TITLE: Expression and purification of recombinant sars coronavirus m protein
INVENTOR(S): Jiang, Weihong; Zhang, Xiaoli; Wang, Jingru; Xie, Youhua; Wang, Yuan; Yang, Sheng
PATENT ASSIGNEE(S): Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Peop. Rep. China
SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, 26 pp.
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1597964	A	20050323	CN 2003-159428	20030917

PRIORITY APPLN. INFO.: CN 2003-159428 20030917

AB The recombinant membrane glycoprotein M of SARS coronavirus is cloned and expressed in E. coli. The method comprises inserting SARS virus M protein gene into vector pMAL-cRI to obtain expression vector. The expression vector is transformed into E.coli. The transformant is cultured at 37±2 °c under inducing with IPTG for 2±0.5 h when OD=0.6±0.2 to express M protein. The recombinant SARS virus M protein is separating and purified on affinity chromatog. column. The invention relates to a **fusion protein** containing SARS virus M protein, maltose binding protein (MBP) and MxeGyrA intein **CBD**. The invention also relates to drug composite containing the above M protein for treatment of SARS.

L3 ANSWER 124 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:218271 CAPLUS
DOCUMENT NUMBER: 142:480879
TITLE: Column flow reactor using acetohydroxyacid synthase I from Escherichia coli as catalyst in continuous synthesis of R-phenylacetyl carbinol
AUTHOR(S): Engel, Stanislav; Vyazmensky, Maria; Berkovich, Dvora; Barak, Ze'ev; Merchuk, Jose; Chipman, David M.
CORPORATE SOURCE: Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Beer Sheva, 84105, Israel
SOURCE: Biotechnology and Bioengineering (2005), 89(6), 733-740
CODEN: BIBIAU; ISSN: 0006-3592
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 142:480879
AB We tested the possibility of utilizing acetohydroxyacid synthase I (AHAS I) from Escherichia coli in a continuous flow reactor for production of R-phenylacetyl carbinol (R-PAC). We constructed a fusion of the large, catalytic subunit of AHAS I with a cellulose binding domain (**CBD**). This allowed purification of the enzyme and its immobilization on cellulose in a single step. After immobilization, AHAS I is fully active and can be used as a catalyst in an R-PAC production unit, operating either in batch or continuous mode. We propose a simplified mechanistic model that can predict the product output of the AHAS I-catalyzed reaction. This model should be useful for optimization and scaling up of a R-PAC production unit, as demonstrated by a column flow reactor.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 125 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:518562 CAPLUS
DOCUMENT NUMBER: 143:187663
TITLE: Development of an inducible suicide gene system based on human caspase 8
AUTHOR(S): Carlotti, Francoise; Zaldumbide, Arnaud; Martin, Patrick; Boulukos, Kim E.; Hoeben, Rob C.; Pognonec, Philippe
CORPORATE SOURCE: UMR 6548 -Universite de Nice, Nice, Fr.
SOURCE: Cancer Gene Therapy (2005), 12(7), 627-639
CODEN: CGTHEG; ISSN: 0929-1903
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Suicide gene-therapy strategies are promising approaches in treating various diseases such as cancers, atherosclerosis, and graft-vs.-host-disease. Here, the authors describe the development of a new effector gene based on inducing functional caspase 8, the initiator caspase in the death-receptor pathway. The authors constructed vectors encoding a constitutively active form of human caspase 8 (CC8), and demonstrated the efficient killing of a variety of cell types in transfection and lentivirus-transduction assays. The authors then analyzed the ability to control the apoptotic activity of a caspase 8-derived construct through the ARIAD homodimerization system (FKC8), a system shown to be extremely effective in several cellular models upon retroviral and lentiviral gene transfer. Similarly, two transcription-regulation systems, muristerone-regulated and Tet-On, were tested to control the expression of CC8. The homodimerization-regulated system FKC8 was shown to be the most efficient system with low background activity in noninduced conditions. In the presence of a dimerizer, it was as active as the activated Tet-On system. From these data, the authors

conclude that the dimerizer-dependent human caspase 8 represents a highly inducible and very powerful system to eradicate transduced cell populations. In addition to its application in exptl. gene therapy, this variant may be highly useful for mechanistic research related to apoptosis.

REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 126 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:1227528 CAPLUS
TITLE: Synthesization, expression, purification and activity assay of hIGF-1
AUTHOR(S): Gao, Yuan; Yu, Rongjie; Hong, An; Li, Zhiying
CORPORATE SOURCE: Bioengineering Institute, Jinan University, Guangzhou, Guangdong Province, 510632, Peop. Rep. China
SOURCE: Zhongguo Bingli Shengli Zazhi (2005), 21(2), 260-265
CODEN: ZBSZEB; ISSN: 1000-4718
PUBLISHER: Jinan Daxue
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB The synthesized human insulin like growth factor I (hIGF-1) gene was expressed in Escherichia coli with high expression level. The means for increasing the efficiency of factor Xa cleavage on purification of hIGF-1 were also discussed. The gene of hIGF-1 was designed and synthesized according to the preference of E. coli. A **fusion protein** with a recognized site of factor Xa between **CBD** (cellulose binding domain) and hIGF-1 was expressed and purified by cellulose affinity chromatog. MTT method was used to assay the bioactivity of **CBD** -IGF **fusion protein**. In order to improve the sensitivity of **fusion protein** to factor Xa, the short flexible peptide (Gly-Thr-Gly-Gly-Gly-Ser-Gly) was added before the recognized site of factor Xa. SDS-PAGE results indicated that the **CBD**-IGF **fusion protein** was expressed and purified. Biol. assay results indicated **CBD**-IGF **fusion protein** could promote the growth of NIH3T3 cell. The short flexible peptide (Gly-Thr-Gly-Gly-Gly-Ser-Gly), which was added before the recognized site of factor Xa, improved the sensitivity of **fusion protein** to factor Xa. **CBD**-IGF **fusion protein** with bioactivity was successfully expressed and purified from E. coli. The short flexible peptide (Gly-Thr-Gly-Gly-Gly-Ser-Gly) could improve the cleavage efficiency of factor Xa.

L3 ANSWER 127 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:182368 CAPLUS
DOCUMENT NUMBER: 140:229401
TITLE: Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands
INVENTOR(S): Come, Jon H.; Becker, Frank; Kley, Nikolai A.; Reichel, Christoph
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 238 pp., Cont.-in-part of U.S. Ser. No. 91,177.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004043388	A1	20040304	US 2002-234985	20020903
US 2003165873	A1	20030904	US 2002-91177	20020304
US 2004266854	A1	20041230	US 2004-820453	20040407

PRIORITY APPLN. INFO.: US 2001-272932P P 20010302
US 2001-278233P P 20010323
US 2001-329437P P 20011015
US 2002-91177 A2 20020304
US 2001-336962P P 20011203
WO 2002-US6677 A2 20020304
US 2002-234985 A2 20020903
WO 2002-US33052 A2 20021015
US 2003-460921P P 20030407
US 2003-531872P P 20031223

AB The invention provides compns. and methods for isolating ligand-binding polypeptides for a user-specified ligand, and for isolating small mol. ligands for a user-specified target polypeptide using an improved class of hybrid ligand compds. Preparation of compds., e.g a methotrexate moiety linked by a polyethylene gycol moiety to dexamethasone, is described.

L3 ANSWER 128 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:120564 CAPLUS
DOCUMENT NUMBER: 140:177854
TITLE: Metal ion-affinity peptides and method for purification of recombinant proteins
INVENTOR(S): Hernan, Ronald A.; Mehigh, Richard J.; Brockie, Ian R.; Jenkins, Elizabeth
PATENT ASSIGNEE(S): Sigma-Aldrich Co., USA
SOURCE: U.S. Pat. Appl. Publ., 37 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004029781	A1	20040212	US 2003-460524	20030612

PRIORITY APPLN. INFO.: US 2002-388059P P 20020612
OTHER SOURCE(S): MARPAT 140:177854

AB The present invention generally relates to the expression and purification of recombinant polypeptides, containing a metal ion-affinity peptide, as well as to genes coding for such polypeptides, expression vectors and transformed microorganisms containing such genes. This invention describes a process for separating a **fusion protein** or polypeptide in the form of its precursor from a mixture containing said **fusion protein** and impurities, which comprises contacting said **fusion protein** with a resin containing immobilized metal ions, said **fusion protein** covalently operably linked directly or indirectly to an immobilized metal ion-affinity peptide, binding said **fusion protein** to said resin, and selectively eluting said **fusion protein** from said resin. The recombinant polypeptides of the present invention are defined by the general formula R1-Sp1-(His-Z1-His-Arg-His-Z2-His)-Sp2-R2 (His-Z1-His-Arg-His-Z2-His = metal ion-affinity peptide; R1,R2 = H, a polypeptide, protein or protein fragment; Sp1,Sp2 = covalent bond, spacer comprising at least one amino acid residue; Z1 = amino acid residue selected from Ala, Arg, Asn, Asp, Gln, Glu, Ile, Lys, Phe, Pro, Ser, Thr, Trp, Val; Z2 = amino acid selected from Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Ile, Leu, Lys, Met, Pro, Ser, Thr, Tyr, Val). Thus, for example, R1 or R2 may comprise a target polypeptide, protein, or protein fragment which is directly or indirectly linked to the metal ion-affinity peptide. Examples of possible spacer peptides and their sequences were provided. Construction and screening of a metal ion-affinity peptide library were demonstrated. Metal ion-affinity **fusion protein** purification protocols were provided.

L3 ANSWER 129 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:18840 CAPLUS
DOCUMENT NUMBER: 140:88703
TITLE: Construction and manufacture of modified **CBD** /RGD recombinant attachment factor for improving cell-attachment efficiency
INVENTOR(S): Lin, Chia-Hui
PATENT ASSIGNEE(S): Bio999 Inc., Taiwan
SOURCE: U.S. Pat. Appl. Publ., 12 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004005690	A1	20040108	US 2002-180120	20020627
EP 1382681	A1	20040121	EP 2002-90251	20020715
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
PRIORITY APPLN. INFO.:			US 2002-180120 A	20020627

AB The present invention relates to a method for improving cell attachment efficiency, especially to a modified **CBD**/RGD recombinant attachment factor for improving cell attachment efficiency. The present invention provides a **CBD**/RGD recombinant attachment factor with improved cell-attachment efficiency, which enables the Arg-Gly-Asp (RGD) amino acid sequence for controlling the cell-attachment to be grafted on the C-terminal having a cellulose binding domain (**CBD**). Thus, by the **CBD**-RGD polypeptide, the cell can be enabled to attach onto the cellulose culturing plate, and as an RGD sequence is added, it can be accommodated in a stable annular structure built up by disulfide bonds, and is grafted on the N-terminal with the **CBD**, so as to form a **CBD**/RGD recombinant attachment factor for enhancing the promotion capability of cell-attachment conspicuously.

L3 ANSWER 130 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:654436 CAPLUS
DOCUMENT NUMBER: 143:243858
TITLE: Modified pituitary protein PACAP (adenylate cyclase-activating peptide) derived thioester (PDT) and therapeutic use
INVENTOR(S): Hong, An; Yu, Rongjie
PATENT ASSIGNEE(S): Jinan University, Peop. Rep. China
SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, No pp. given
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1557835	A	20041229	CN 2004-10015136	20040115
PRIORITY APPLN. INFO.:			CN 2004-10015136	20040115

AB The present invention provides novel adenylate cyclase-activating peptide derived thioester (PDT) that are chemical modified at C-terminal with β-mercaptoethanol. Compared with natural and chemical synthesized adenylate cyclase activating polypeptide, PDT retains the same activity with increased stability.

L3 ANSWER 131 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:158210 CAPLUS
DOCUMENT NUMBER: 140:355894
TITLE: Improvement of Cellulose-Degrading Ability of a Yeast Strain Displaying Trichoderma reesei Endoglucanase II by Recombination of Cellulose-Binding Domains
AUTHOR(S): Ito, Junji; Fujita, Yasuya; Ueda, Mitsuyoshi; Fukuda, Hideki; Kondo, Akihiko
CORPORATE SOURCE: Department of Chemical Science and Engineering Faculty of Engineering and Division of Molecular Science Graduate School of Science and Technology, Kobe University, Kobe, 657-8501, Japan
SOURCE: Biotechnology Progress (2004), 20(3), 688-691
CODEN: BIPRET; ISSN: 8756-7938
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To improve the cellulolytic activity of a yeast strain displaying endoglucanase II (EGII) from the filamentous fungus Trichoderma reesei QM9414, the genes encoding the cellulose-binding domain (**CBD**) of EGII, cellobiohydrolase I (CBHI) and cellobiohydrolase II (CBHII) from T. reesei QM9414, were fused with the catalytic domain of EGII and expressed in Saccharomyces cerevisiae. Display of each of the recombinant EGIIs was confirmed using immunofluorescence microscopy. In the case of EGII-displaying yeast strains in which the **CBD** of EGII was replaced with the **CBD** of CBHI or CBHII, the binding affinity to Avicel and hydrolytic activity toward phosphoric acid swollen Avicel were similar to that of a yeast strain displaying wild-type EGII. On the other hand, the three yeast strains displaying EGII with two or three tandemly aligned **CBDs** showed binding affinity and hydrolytic activity higher than that of the yeast strain displaying wild-type EGII. This result indicates that the hydrolytic activity of yeast strains displaying recombinant EGII increases with increased binding ability to cellulose.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 132 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:363192 CAPLUS
DOCUMENT NUMBER: 141:105304
TITLE: Increased antifungal and chitinase specific activities of Trichoderma harzianum CECT 2413 by addition of a cellulose binding domain
AUTHOR(S): Limon, M. C.; Chacon, M. R.; Mejias, R.; Delgado-Jarana, J.; Rincon, A. M.; Codon, A. C.; Benitez, T.
CORPORATE SOURCE: Facultad de Biologia, Departamento de Genetica, Universidad de Sevilla, Seville, 41080, Spain
SOURCE: Applied Microbiology and Biotechnology (2004), 64(5), 675-685
CODEN: AMBIDG; ISSN: 0175-7598
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Trichoderma harzianum is a widely distributed soil fungus that antagonizes numerous fungal phytopathogens. The antagonism of T. harzianum usually correlates with the production of antifungal activities including the secretion of fungal cell walls that degrade enzymes such as chitinases. Chitinases Chit42 and Chit33 from T. harzianum CECT 2413, which lack a chitin-binding domain, are considered to play an important role in the biocontrol activity of this strain against plant pathogens. By adding a cellulose-binding domain (**CBD**) from cellobiohydrolase II of Trichoderma reesei to these enzymes, hybrid chitinases Chit33-**CBD** and Chit42-**CBD** with stronger chitin-binding capacity than the native chitinases have been engineered. Transformants that overexpressed

the native chitinases displayed higher levels of chitinase specific activity and were more effective at inhibiting the growth of Rhizoctonia solani, Botrytis cinerea and Phytophthora citrophthora than the wild type. Transformants that overexpressed the chimeric chitinases possessed the highest specific chitinase and antifungal activities. The results confirm the importance of these endochitinases in the antagonistic activity of T. harzianum strains, and demonstrate the effectiveness of adding a **CBD** to increase hydrolytic activity towards insol. substrates such as chitin-rich fungal cell walls.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 133 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:643663 CAPLUS
DOCUMENT NUMBER: 144:268153
TITLE: The expression and purification of enhanced green fluorescent protein mutant mut4EGFP in E.coli
AUTHOR(S): Niu, Chuanshuang; Fang, Liurong; Xiao, Shaobo; Zhang, Hui; Chen, Huanchun
CORPORATE SOURCE: College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, Peop. Rep. China
SOURCE: Huazhong Nongye Daxue Xuebao (2004), 23(5), 489-491
CODEN: HNDXEK; ISSN: 1000-2421
PUBLISHER: Huazhong Nongye Daxue Xuebao Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Mut4EGFP, a novel enhanced green fluorescent protein mutant (EGFP/V163A/S175G), is more bright and stable than EGFP. In order to obtain the purified protein, the DNA fragment encoding mut4EGFP was inserted into prokaryotic expression vector pTWIN1, resulting in the expression plasmid pTWIN-M4, pTWIN-M4 was further transformed into BL21(DE3)plysS, induced by IPTG and expression was analyzed using SDS-PAGE. The results showed that mut4EGFP was expressed and existed in soluble form. The expressed products were further purified using chitin beads and mg-grade recombinant proteins were obtained.

L3 ANSWER 134 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:582855 CAPLUS
DOCUMENT NUMBER: 141:237660
TITLE: Gene synthesis, expression of pituitary adenylate cyclase activating polypeptide and its purification and identification
AUTHOR(S): Yu, Rong-jie; Hong, An; Zhang, Ling; Zhou, Tian-hong; Dai, Yun; Gao, Yuan
CORPORATE SOURCE: Bio-engineering Institute, Jinan University, Guangzhou, 510632, Peop. Rep. China
SOURCE: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (2004), 20(3), 376-382
CODEN: ZSHXF2; ISSN: 1007-7626
PUBLISHER: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB To produce pituitary adenylate cyclase activating polypeptide (PACAP) using gene engineering technol., a gene coding PACAP was designed and synthesized according to the preference of E. coli, and cloned into the expression vector pET-35b(+). The recombinant plasmid pET-PACAP were constructed and transformed into E. coli BL21(DE3)pLysS+. A **fusion protein** with a recognized site of factor Xa between **CBD** (cellulose binding domain) and PACAP was expressed and purified by cellulose affinity chromatog. PACAP was released by the cleavage of factor Xa. A short flexible peptide (Gly-Thr-Gly-Gly-Gly-Ser-Gly), was added before the recognized site by factor Xa to improve

the sensitivity of **fusion protein** to factor Xa.
PACAP with over 95% purity was purified by HPLC and identified by Western blotting. Laser time-of-flying mass spectrum showed that the mol. weight was 4536.8 as expected. The preliminary bioactivity assay indicated that the product had the activity promoting cAMP synthesis in the cell line SW1990 of human pancreas carcinoma.

L3 ANSWER 135 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:285751 CAPLUS
DOCUMENT NUMBER: 144:47082
TITLE: A new strategy for improving the cleavage efficiency of factor Xa
AUTHOR(S): Yu, Rongjie; Gao, Yuan; Hong, An
CORPORATE SOURCE: Bio-engineering Institute, Jinan University, Guangzhou, 510632, Peop. Rep. China
SOURCE: Zhongguo Shengwu Gongcheng Zazhi (2004), 24(12), 84-88
CODEN: ZSGZAW; ISSN: 1671-8135
PUBLISHER: Zhongguo Shengwu Gongcheng Zazhishe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB In order to improve the cleavage efficiency of factor Xa on **fusion proteins CBD**-IGF and **CBD**-PACAP and produce non-fusion recombinant peptides efficiently, a short flexible peptide abundant in Gly (Gly-Thr-Gly-Gly-Gly-Ser-Gly) was added before the recognized site by factor Xa using gene engineering technol. All **fusion proteins** were purified by cellulose-affinity chromatog. The compare of the cleavage efficiency of factor Xa on both two group **fusion proteins** indicated that the short peptide helped to improve the sensitivity of **fusion protein** to factor Xa to different degrees. The sensitivities of **CBD**-IGF **fusion proteins** were lower than that of **CBD**-PACAP **fusion proteins** as a whole. A strategy of promoting the cleavage efficiency of factor Xa was presented.

L3 ANSWER 136 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:719641 CAPLUS
DOCUMENT NUMBER: 139:256271
TITLE: Microarrays using soluble cellulose binding chimeric proteins for immobilization and methods of use thereof
INVENTOR(S): Morag, Ely
PATENT ASSIGNEE(S): Zephyr Proteomix Ltd., Israel
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003074722	A2	20030912	WO 2003-IL177	20030306
WO 2003074722	A3	20050630		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2478298	AA	20030912	CA 2003-2478298	20030306

AU 2003219477	A1	20030916	AU 2003-219477	20030306
EP 1565744	A2	20050824	EP 2003-715289	20030306
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, SK				
US 2005118729	A1	20050602	US 2004-933404	20040903
PRIORITY APPLN. INFO.:			US 2002-362061P	P 20020307
			WO 2003-IL177	W 20030306

AB The present invention provides microarrays of proteins comprising a cellulose binding domain (**CBD**) as a means for binding to a cellulase substrate such as cellulose. The cellulose binding region may further contain biol. active exogenous moieties introduced therein while maintaining the ability to bind cellulase substrates with high affinity. In addition, methods of construction of the microarrays and using said microarrays are disclosed. The microarrays of the invention are particularly useful for displaying peptide libraries, random or rationally designed, and for high throughput screening for ligands, epitopes or ligand binding sites. In particular, the 168-amino acid soluble **CBD** of the scaffoldin subunit S1 from the cellulosome of Clostridium thermocellum fused with a N-terminal 38-amino acid flanking peptide and a 34-amino acid C-terminal flexible hinge linker are designed to microarray immobilization. Also disclosed are plasmid construction, expression and purification for **fusion proteins**, termed ZEPHYRIN41 (HIV type 1 only) and ZEPHYRIN36 (for HIV type 2 only) and ZEPHYRIN120 (for HIV type 1 and 2), of the above recombinant **CBD** protein and HIV antigenic peptides. Furthermore, antigen-peptide microarray for serodiagnosis of HIV type 1 and type 2 was prepared using the above recombinant proteins, which allows simultaneous detection of both antibodies directed against HIV type 1 and antibodies directed against HIV type 2. Also provided are biotinylated **CBDs** (at Cys55), which allows for efficient binding of biotin-binding mols., e.g. avidin or streptavidin, to cellulose and the resultant matrix is appropriate for use as a universal affinity system.

L3 ANSWER 137 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER:	2003:238757 CAPLUS
DOCUMENT NUMBER:	140:90031
TITLE:	Synthesis of protein-nucleic acid conjugates by expressed protein ligation
AUTHOR(S):	Lovrinovic, Marina; Seidel, Ralf; Wacker, Ron; Schroeder, Hendrik; Seitz, Oliver; Engelhard, Martin; Goody, Roger S.; Niemeyer, Christof M.
CORPORATE SOURCE:	Fachbereich Chemie, Biologisch-Chemische Mikrostrukturtechnik, Universitaet Dortmund, Dortmund, D-44227, Germany
SOURCE:	Chemical Communications (Cambridge, United Kingdom) (2003), (7), 822-823 CODEN: CHCOFS; ISSN: 1359-7345
PUBLISHER:	Royal Society of Chemistry
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB The synthesis of covalent conjugates of proteins and polyamide nucleic acids (PNA) is accomplished by expressed protein ligation of intein-**fusion proteins** and a PNA-cysteine conjugate.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 138 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER:	2003:621678 CAPLUS
DOCUMENT NUMBER:	140:249420
TITLE:	Use of micro-porous affinity membranes for protein purification: A case study
AUTHOR(S):	Cattoli, F.; Sarti, G. C.
CORPORATE SOURCE:	DICMA - Dipartimento di Ingegneria Chimica, Mineraria

e delle Tecnologie Ambientali, Alma Mater Studiorum - Universita di Bologna, Bologna, 40136, Italy
SOURCE: Membrane Science and Technology Series (2003), 8(New Insights into Membrane Science and Technology: Polymeric and Biofunctional Membranes, 2003), 263-281
CODEN: MSSREV; ISSN: 0927-5193
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The recovery of MBP-intein-**CBD** using amylose affinity membranes has been studied. MBP-intein-**CBD** is a model **fusion protein** containing three different protein domains: MBP as the affinity target, **CBD** as the protein of interest and the intein domain inserted between MBP and **CBD** in view of its self-cleavage activity. Such a **fusion protein** allows for the recovery of the target product in a single chromatog. step. The affinity separation process is conducted exploiting the specific interaction of the MBP group towards amylose. The use of micro-porous membranes as stationary phase is considered in order to improve the fluid dynamic of the system, i.e. flow rate, pressure drop and mass transport in the liquid phase. A large scale membrane holder has been used, suitable for the separation of large amts. of the desired product. Good results in terms of purity and concentration of the product were achieved. Kinetic and equilibrium data have been obtained; the exptl. equilibrium curve was well represented by the Langmuir isotherm; the kinetic consts. were determined based on a simplified kinetic model.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 139 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:940379 CAPLUS
DOCUMENT NUMBER: 140:109782
TITLE: A Human Synthetic Combinatorial Library of Arrayable Single-chain Antibodies based on Shuffling in Vivo Formed CDRs into General Framework Regions
AUTHOR(S): Azriel-Rosenfeld, Ronit; Valensi, Moran; Benhar, Itai
CORPORATE SOURCE: The George S. Wise Faculty of Life Sciences, Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Ramat Aviv, 69978, Israel
SOURCE: Journal of Molecular Biology (2003), Volume Date 2004, 335(1), 177-192
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We describe a novel approach for high-throughput screening of recombinant antibodies, based on their immobilization on solid cellulose-based supports. We constructed a large human synthetic single-chain Fv antibody library where in vivo formed complementarity determining regions were shuffled combinatorially onto germline-derived human variable-region frameworks. The arraying of library-derived scFvs was facilitated by our unique display/expression system, where scFvs are expressed as **fusion proteins** with a cellulose-binding domain (**CBD**). Escherichia coli cells expressing library-derived scFv-**CBDs** are grown on a porous master filter on top of a second cellulose-based filter that captures the antibodies secreted by the bacteria. The cellulose filter is probed with labeled antigen allowing the identification of specific binders and the recovery of the original bacterial clones from the master filter. These filters may be simultaneously probed with a number of antigens allowing the isolation of a number of binding specificities and the validation of specificity of binders. We screened the library against a number of cancer-related peptides, proteins, and peptide-protein complexes

and yielded antibody fragments exhibiting dissociation consts. in the low nanomolar range. We expect our new antibody phage library to become a valuable source of antibodies to many different targets, and to play a vital role in facilitating high-throughput target discovery and validation in the area of functional cancer genomics.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 140 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:906480 CAPLUS
DOCUMENT NUMBER: 138:12383
TITLE: Engineered biotin ligase BirA for in vitro biotinylation of recombinant protein to facilitate purification
INVENTOR(S): Wong, Sui-lam; Wu, Sau-ching
PATENT ASSIGNEE(S): University Technologies International, Inc., Can.
SOURCE: PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002095013	A2	20021128	WO 2002-CA760	20020524
WO 2002095013	A3	20040115		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2346220	AA	20021124	CA 2001-2346220	20010524
PRIORITY APPLN. INFO.:			CA 2001-2346220 A	20010524

AB An engineered biotin ligase (**CBD**-BirA-His) includes a chitin binding domain tag and a polyhistidine tag. The engineered BirA contains 53-AA(amino acid) chitin binding domain followed by a 18-AA peptide linker at the N-terminal of the biotin ligase and a hexahistidine at the C-terminal of the biotin ligase. The engineered BirA may be used to biotinylate proteins such as staphylokinase in a protein purification scheme. Purified engineered BirA demonstrates high biol. activity and is active in a fairly broad pH range. The invention also demonstrates that functional staphylokinase (a very promising blood clot dissolving agent, with a 15-AA biotinylation tag attached at the C-terminus) could be purified via in vitro biotinylation using **CBD**-BirA-His.

L3 ANSWER 141 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:461306 CAPLUS
DOCUMENT NUMBER: 137:29042
TITLE: Preparation of multifunctional chimeric proteins with a cellulose binding domain and uses thereof
INVENTOR(S): Chen, David Chanhan; Hu, Nien-tai; Chen, Yun-ju; Hseu, Tzong-hsiung
PATENT ASSIGNEE(S): Taiwan
SOURCE: U.S., 17 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6407208	B1	20020618	US 1998-166966	19981006
TW 552268	B	20030911	TW 1997-86114750	19971008
PRIORITY APPLN. INFO.:			TW 1997-86114750 A	19971008

AB The present invention discloses method of preparation of multifunctional chimeric proteins with a cellulose binding domain by genetic recombinant techniques, wherein two exogenous bifunctional amino acid sequences are resp. joined at the C-terminal and the N-terminal of the cellulose binding domain of cellulase. In addition to its affinity to cellulose due to the presence of the **CBD**, various properties can be adopted to the chimeric protein by means of introducing the desired proteins upstream and downstream of the **CBD**. Specifically, the invention provides a chimeric protein that is produced in Escherichia coli in large quantities by joining a thioredoxin, a **CBD** and a short peptide composed of 3 amino acid (i.e., Arginine-Glutamate-Asparate). This chimeric protein can be further purified in simple steps and added to cell culture to enhance cell attachment to a surface made up of cellulose.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 142 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:126201 CAPLUS
DOCUMENT NUMBER: 136:196191
TITLE: Stain or textile fiber composition binding domain-containing chimeric enzymes for use in detergent
INVENTOR(S): Shimotsuura, Isao; Tobe, Seiichi
PATENT ASSIGNEE(S): Lion Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 39 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002051768	A2	20020219	JP 2000-244723	20000811
PRIORITY APPLN. INFO.:			JP 2000-244723	20000811

AB Chimeric enzymes comprising a peptide having affinity for stain or textile fiber composition and an enzyme having the similar affinity, including non-cellulolytic enzymes, are disclosed. Peptides having affinity for polysaccharides, proteins, or lipids, such as mutase mutein binding domain, amylase starch-binding domain, glucan-binding domain of β-glucosidase or glucosyltransferase, glucan-binding protein, chitin-binding protein, β-1,3-glucan-binding protein, cellulose-binding protein, lectin, cellulase, xylanase, mannase, chitinase, and other polysaccharide-degrading enzyme and cellulose-binding domain (**CBD**)-containing proteins of family III, IV, VII, VIII, IX, or X are used. A peptide from proteins involved in keratin biosynthesis or degradation, or keratin binding domain of an enzyme can also be used. Detergent containing the chimeric enzyme is claimed. Preparation of chimeric enzymes comprising alkaline protease, amylase, lipase, pectinase, laccase, peroxidase, and glucose oxidase with peptides mentioned above, and demonstration of improved cleaning power as detergent composition, are described.

L3 ANSWER 143 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:972914 CAPLUS

DOCUMENT NUMBER: 141:3483
TITLE: Cellulose-binding **fusion proteins**
AUTHOR(S): Boraston, Alisdair B.; McLean, Bradley W.; Kavoosi, Mojgan; Haynes, Charles A.; Kilburn, Douglas G.
CORPORATE SOURCE: Switz.
SOURCE: Methods for Affinity-Based Separations of Enzymes and Proteins (2002), 148-162. Editor(s): Gupta, Munishwar Nath. Birkhaeuser Verlag: Basel, Switz. CODEN: 69EWCN; ISBN: 3-7643-6305-3
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The use of cellulose binding domains (**CBDs**) to confer specific adhesives properties on proteins appears to have widespread applicability. **CBDs** can be coupled by chemical or mol. genetic techniques and, in the latter case, can be located at either terminal of a target protein. This is significant if the presence of a **CBD** on one of the termini inactivates the protein. Cellulose-binding **fusion proteins** in general retain the activity of both the **CBD** and the coupled partner. Methods are described for the purification of the β-glucosidase-CBDCex and green fluorescence protein-CBDIX fusion from cell cultures.
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 144 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:380488 CAPLUS
DOCUMENT NUMBER: 137:211590
TITLE: Production of a recombinant antimicrobial peptide in transgenic plants using a modified VMA intein expression system
AUTHOR(S): Morassutti, Carla; De Amicis, Francesca; Skerlavaj, Barbara; Zanetti, Margherita; Marchetti, Stefano
CORPORATE SOURCE: DPVTA, Universita di Udine, Udine, 33100, Italy
SOURCE: FEBS Letters (2002), 519(1-3), 141-146 CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Tobacco plants were engineered to express SMAP-29, a mammalian antimicrobial peptide of innate immunity, as **fusion protein** with modified vacuolar membrane ATPase intein. The peptide was purified taking advantage of the intein-mediated self-cleaving mechanism. SMAP-29 was immunol. detected in the chromatog. eluate and appeared tightly bound to copurified plant proteins. Electrophoretic separation under disaggregating conditions indicated that the recombinant peptide was cleaved off by intein at the expected site and an overlay gel assay demonstrated that the peptide retained antimicrobial activity. These results indicate that a modified intein expression system can be used to produce pharmaceutical peptides in transgenic plants.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 145 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:186510 CAPLUS
TITLE: Modulation of wood fibers and paper by cellulose binding domains (**CBDs**)
AUTHOR(S): Shoseyov, Oded; Shani, Z.; Levi, Ilan; Mansfield, Shawn
CORPORATE SOURCE: Kennedy Leigh Centre for Horticulture Research, Hebrew University of Jerusalem, Rehovot, 76100, Israel
SOURCE: Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002 (2002), CELL-100. American Chemical Society: Washington, D.

C.
CODEN: 69CKQP
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Recombinant cellulose-binding domain (**CBD**) has previously been shown to modulate the elongation of different plant cells in vitro (Shpigel et. al. , 1988). Using Acetobacter xylinum as a model system, **CBD** was found to increase the activity of the cellulose synthase enzyme in a dose-dependent manner, up to fivefold, as compared with the control untreated cells. Introduction of the **CBD** gene under the control of the elongation specific cell promoter (Shani et. al. , 1997) into transgenic poplar plants led to significant increases in biomass production in selected clones, as compared with wild type control plants. Anal. of wood characteristics from transgenic poplar trees showed significant increases in fiber cell length, as well as an increase in the average d.p. of cellulose. In addition, a significant decrease in microfibril angle was observed These results coincided with increased burst, tear and tensile indexes of paper prepared from these wood fibers. The mechanism by which **CBD** affects cell wall metabolism remains unknown, but we postulate a physico-mech. mechanism, whereby **CBD** slides between cellulose fibers and separates them in a wedge-like action. This postulate is supported by in vitro experimentation: application of recombinant **CBD** significantly reduces the wet-tensile strength of cellulose paper as tested by instron, thus resembling the in-vitro effect of expansin. Furthermore, petunia cell suspensions, treated with increasing concns. of **CBD** displayed an abnormal shedding of cell wall layers, indicating that **CBD** has the potential to cause non-hydrolytic cell wall disruption activity in-vivo. **CBD fusion proteins** may be used to crosslink and introduce functional mols. to paper materials. The applications of such mols. will be siscussed. Shpigel et. al. , 1988, Plant Physiol. 117: 1185-1194. Shani et. al. , 1997 Plant Mol.Biol. 34: 837-842.

L3 ANSWER 146 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:186509 CAPLUS
TITLE: Characterisation and expression in-planta of a fungal cellulose-binding domain
AUTHOR(S): Quentin, Michael; Derksen, Jan; DeJong, Ed; Mariani, Celestina; VanderValk, Henry
CORPORATE SOURCE: Department of Fibre & Paper Technology, ATO BV, Wageningen, 6700 AA, Neth.
SOURCE: Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002 (2002), CELL-099. American Chemical Society: Washington, D. C.
CODEN: 69CKQP
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Fiber surface structure plays a key role in fiber properties, and their chemical composition depends on the raw material and on the production process. Targetted enzymic modification of the fiber surface may lead to improved tech. qualities and processibility of the fibers. In addition, modification of the biosynthesis and assembly of cell walls of fiber-yielding plants is of great interest in optimizing requested fibers properties. Most cellulases of microbial origine have a three-domain structure consisting of a catalytic domain, a cellulose-binding domain (**CBD**), separated by a distinct linker region. The **CBD** seems to be responsible for targetting and for facilitating the activity of the catalytic domain on insol. cellulosic substrate. **CBDs** are stucturally and functionally independent from the catalytic domain; and as they can be used to construct **fusion proteins** without affecting the biol. activity of the hybrid (1), they represent interesting tools for fiber surface modification. Bacterial **CBDs** have also been shown

to enhance plant development and cellulose yield when expressed in plants (2), and could be used to modify fibers quality in-planta. A cellulose-binding domain and a serine- and threonine-rich linker peptide were cloned from the fungi Aspergillus japonicus and Aspergillus aculeatus. A **fusion protein** GST-**CBD**, in which the glutathione S-transferase was linked to the peptide linker and to the cellulose-binding domain at its C-terminus, was expressed in Escherichia coli. Renaturated GST-**CBD**, recovered from inclusion bodies and purified on Glutathione Sepharose, adsorbed to both bacterial microcryst. cellulose and CM-cellulose. Deletion of the linker peptide affected the ability of the hybrid protein to adsorb to cellulose, and made it more sensitive to protease digestion. The sequences coding for this fungal cellulose-binding domain was introduced in Arabidopsis thaliana via Agrobacterium thumefaciens. Plants expressing the cellulose-binding domain were shown to be affected in their development. Ong et. al. 1989. Trends in Biotechnol. Vol.7. pp.239-243. Shpigel et. al. 1998. Plant Physiol. Vol.117. pp.1185-1194.

L3 ANSWER 147 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:178717 CAPLUS
DOCUMENT NUMBER: 137:126634
TITLE: Effects of a cellulose binding domain on deinking of recycled mixed office paper
AUTHOR(S): Li, Kaichang; Xu, Xia
CORPORATE SOURCE: Department of Wood Science and Engineering, Oregon State University, Corvallis, OR, 97331, USA
SOURCE: Progress in Paper Recycling (2002), 11(2), 9-13
CODEN: PPREFY; ISSN: 1061-1452
PUBLISHER: Doshi & Associates Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A **fusion protein** containing a cellulose binding domain (**CBD**) from Cellulomonas fimi endoglucanase A was prepared and purified. The purified **CBD** protein was used in the deinking of a mixed office paper (MOP). The deinking process included the re-pulping of the MOP, incubation of MOP pulp slurry with the **CBD** protein, and a flotation. It was shown that the incubation of the pulp slurry with the **CBD** greatly increased total dirt count and residual ink area. Direct addition of the **CBD** to the flotation stage also increased dirt count and residual ink area significantly. The overall decrease of the deinking efficiency by the **CBD** was caused by a decrease in the efficiency of ink removal in the flotation stage.
REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 148 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:935750 CAPLUS
DOCUMENT NUMBER: 136:50678
TITLE: Methods for immobilizing polypeptides
INVENTOR(S): Nock, Steffen; Sydor, Jens
PATENT ASSIGNEE(S): Zyomyx, Inc., USA
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001098458	A2	20011227	WO 2001-US19531	20010619

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US,
UZ, VN, YU, ZA, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2001069906	A5	20020102	AU 2001-69906		20010619
US 2002049152	A1	20020425	US 2001-884269		20010619
US 2004058390	A1	20040325	US 2003-669241		20030923
PRIORITY APPLN. INFO.:			US 2000-212620P	P	20000619
			US 2001-884269	A1	20010619
			WO 2001-US19531	W	20010619

AB This invention provides methods for immobilizing polypeptides, for forming arrays of polypeptides arranged on a support, and arrays produced using the methods of the invention. The immobilized polypeptides of the invention are generally in the same orientation, can be full-length and biol. active, and can be readily screened for a desired activity.

L3 ANSWER 149 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:582019 CAPLUS
DOCUMENT NUMBER: 135:167037
TITLE: Preparation of circular or multimeric proteins in vivo or in vitro using the protein splicing functions of intein proteins
INVENTOR(S): Evans, Thomas C.; Xu, Ming-Qun
PATENT ASSIGNEE(S): New England Biolabs, Inc., USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2001057183	A2	20010809	WO 2001-US3147		20010131
WO 2001057183	A3	20020214			
W: JP, US					
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR					
US 2003013148	A1	20030116	US 2002-937070		20020129
PRIORITY APPLN. INFO.:			US 2000-180319P	P	20000204
			WO 2001-US3147	W	20010131

AB A method is disclosed for the in vitro or in vivo cyclization of protein or peptide sequences. Also disclosed is a method of fusing polypeptide sequences while bound to a solid support. These protein manipulation techniques relied on the trans-splicing activity of a split intein, such as the naturally occurring split intein form the dnaE gene of Synechocystis sp. PCC6803 (Ssp DnaE intein). The cyclization procedures required the fusion of C- and N-terminal intein splicing domains to the N- and C-termini, resp., of a target protein (Inteinc-target protein-InteinN). Cyclization in vivo occurred post-translationally when the two complementary intein splicing domains ligated the N- and C-terminus of the target protein. In vitro cyclization also utilized and InteinC-target protein-InteinN precursor protein, in which the intein domains were fused to a chitin binding domain (**CBD**). Protein expression was conducted under conditions that favored the accumulation of precursor protein, which was immobilized on a chitin resin. The circular protein species were eluted from the chitin resin following incubation under conditions that favored protein splicing. Trans-splicing was used to ligate polypeptides on a solid support by generating a protein composed of a **CBD** fused to a C-terminal intein splicing domain and target

protein. This was incubated with a protein composed of target protein fused to an N-terminal intein splicing domain and a **CBD**. The precursor proteins were immobilized on a chitin resin where trans-splicing resulted in the ligation of target protein to target protein. These techniques greatly expand the procedures available for protein engineering and modification.

L3 ANSWER 150 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:320131 CAPLUS
DOCUMENT NUMBER: 134:337624
TITLE: Enzyme immobilization via chitin-cellulose binding domain and use in industrial synthesis of chemicals
INVENTOR(S): Tanaka, Atsuo; Ueda, Mitsuyoshi; Nagao, Koji
PATENT ASSIGNEE(S): Fujisawa Pharmaceutical Co., Ltd., Japan
SOURCE: PCT Int. Appl., 100 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001031038	A1	20010503	WO 2000-JP7275	20001019
W: CA, CN, JP, KR, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
JP 2005095001	A2	20050414	JP 1999-301699	19991022
PRIORITY APPLN. INFO.:			JP 1999-301699 A	19991022

AB A method of immobilizing proteins having a plural number of subunits, on a chitin or cellulose by expressing a protein as fusion with a chitin-cellulose binding domain (**CBD**) by genetic engineering technique, is disclosed. Immobilization of self-splicing proteins, amino acylase or cyclic lipopeptide acylase, in particular, and use in synthesis of certain chemical compds., are claimed. Immobilization of E. coli expressed 7-β-(4-carboxybutanamido)-cephalosporanic acid acylase (C427 GL-7ACA acylase) on a chitin beads via **CBD** is described.
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 151 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:137384 CAPLUS
DOCUMENT NUMBER: 134:188942
TITLE: Genetic system yielding self-cleaving inteins with controllable splicing and/or cleavage activity for bioseparations and methods for determining critical residues for varying cleavage activity
INVENTOR(S): Belfort, Marlene; Belfort, Georges; Derbyshire, Vicky; Wood, David; Wu, Wei
PATENT ASSIGNEE(S): Health Research Institute, USA
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001012820	A1	20010222	WO 2000-US22581	20000817
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK,				

MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000067817	A5	20010313	AU 2000-67817		20000817
US 6933362	B1	20050823	US 2000-641808		20000817
PRIORITY APPLN. INFO.:			US 1999-149257P	P	19990817
			WO 2000-US22581	W	20000817

AB A self-cleaving element for use in biosepns. has been derived from a naturally occurring, 43 kDa protein splicing element (intein) through a combination of protein engineering and random mutagenesis. A mini-intein (18 kDa) previously engineered for reduced size had compromised activity and was therefore subjected to random mutagenesis and genetic selection. In one selection a mini-intein was isolated with restored splicing activity, while in another, a mutant was isolated with enhanced, pH-sensitive C-terminal cleavage activity. The enhanced cleavage mutant has utility in affinity fusion-based protein purification The enhanced splicing mutant has utility in purification of proteins such as toxic proteins, for example, by inactivation with the intein in a specific region and controllable splicing. These mutants also provide new insights into the structural and functional roles of some conserved residues in protein splicing. Thus, disclosed and claimed are: a genetic system and self-cleaving inteins therefrom; biosepns. employing same; protein purification by inactivation with inteins in specific regions and controllable intein splicing; methods for determining critical, generalizable residues for varying intein activity.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 152 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER:	2001:641770 CAPLUS
TITLE:	Affinity membranes for the purification of MBP-**fusion proteins**
AUTHOR(S):	Cattoli, Francesca; Sarti, Giulio C.
CORPORATE SOURCE:	Dipartimento di Ingegneria chimica, mineraria e delle tecnologie ambientali, University of Bologna, 40136 Bologna, Italy
SOURCE:	Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001 (2001), PMSE-274. American Chemical Society: Washington, D. C. CODEN: 69BUZP
DOCUMENT TYPE:	Conference; Meeting Abstract
LANGUAGE:	English

AB An affinity purification process for the class of the MBP-**fusion proteins** is studied, using com. available films, suitably modified into affinity substrates. The affinity membranes are obtained through a chemical modification of a porous matrix formed by native cellulose fibers; amylose has been chosen as ligand. A preparative scale membrane module has been realized suitable to perform sepns. of MBP **fusion proteins** directly from cell lysate. The membrane module set up is suitable for flat sheet membranes and is formed by a series of membrane stages arranged in a column design. On each disk, a stack of membrane can be arranged. The dimension of the module allows the treatment of high vols. of protein mixts. in short processing times. Separation processes of three **fusion proteins** have been performed, i.e. MBP-β-galactosidase, MBP-rubredoxin, and MBP-intein **CBD**. The membrane binding capacity obtained is of the same order of magnitude of the nominal binding capacity existing for com. available supports. The single step process is much faster than for traditional affinity supports and, in addition, leads to protein solns. which are rather concentrated and characterized by a higher purity.

L3 ANSWER 153 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:197454 CAPLUS
TITLE: **CBD**-Factor X **fusion protein** production by a stable transformed Sf9 insect cell line in a high cell density perfusion culture
AUTHOR(S): Gorenflo, Volker M.; Pfeifer, Tom A.; Grigliatti, Thomas A.; Lesnicki, Gary; Kilburn, Doug G.; Piret, James M.
CORPORATE SOURCE: Biotechnology Laboratory & Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Abstracts of Papers, 221st ACS National Meeting, San Diego, CA, United States, April 1-5, 2001 (2001) BIOT-147
CODEN: 69FZD4
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; Meeting Abstract
LANGUAGE: English

AB Factor Xa is a blood coagulation protease, whose selectivity can be used to cleave protein tags from recombinant proteins. A **fusion protein** comprised of a self-activated form of Factor X linked to a cellulose-binding domain, **CBD**-FXa, was produced in a stable transformed Sf9 insect cell line. A 2 L batch fermentation reached a maximum cell concentration of 9 x 106 cells/mL and a final Factor X concentration of 4 mg/L. The production of **CBD**-FXa by this cell line was also analyzed in a 1.5 L perfusion system using an ultrasonic filter as a cell-retention device for flow rates up to 3 L/day. The cell retention efficiency was greater than 95% and Sf9 cell concns. of over 40 x 106 cells/mL with a viability greater than 80 % were obtained. The **CBD**-FXa volumetric productivity of the perfusion system was increased by an order of magnitude in comparison to batch fermns. The influence of different glucose concentration set points and cell concns. were investigated.

L3 ANSWER 154 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:900779 CAPLUS
DOCUMENT NUMBER: 134:52258
TITLE: Recombinant protein expression in plants as cellulose binding peptide **fusion protein** and isolation via affinity binding
INVENTOR(S): Shani, Ziv; Shoseyov, Oded
PATENT ASSIGNEE(S): Cbd Technologies Ltd., Israel; Yissum Research and Development Company of the Hebrew University of Jerusalem; Friedman, Mark, M.
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000077175	A1	20001221	WO 2000-US13434	20000517
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 6331416	B1	20011218	US 1999-329234		19990610
AU 2000051369	A5	20010102	AU 2000-51369		20000517
PRIORITY APPLN. INFO.:			US 1999-329234	A	19990610
			WO 2000-US13434	W	20000517

AB A process of expressing a recombinant protein in a plant and of isolating the recombinant protein from the plant, is disclosed. The process consists of (a) providing a plant, a plant derived tissue or cultured plant cells expressing a **fusion protein** including the recombinant protein and a cellulose binding peptide being fused thereto, the **fusion protein** being compartmentalized within cells of the plant, plant derived tissue or cultured plant cells, so as to be sequestered from cell walls of the cells of the plant, plant derived tissue or cultured plant cells; (b) homogenizing the plant, plant derived tissue or cultured plant cells, so as to bring into contact the **fusion protein** with a cellulosic matter of the plant, plant derived tissue or cultured plant cells, to thereby effect affinity binding of the **fusion protein** via the cellulose binding peptide to the cellulosic matter, thereby obtaining a **fusion protein** cellulosic matter complex; and (c) isolating the **fusion protein** cellulosic matter complex. The recombinant protein can be released from the **fusion protein** by proteolysis at the unique protease recognition sequence. A vector for expression of such **fusion protein** is claimed. Cellulose Binding Domains (**CBDs**) are independently folding protein modules that bind strongly to different forms of cellulose via non-covalent hydrophobic interactions. We have engineered a variety of bifunctional protein fusions which bind to cellulose and retain the function of the fusion partner. These proteins can be expressed in standard systems including microbial, insect, mammalian and plant cells. The presence of the **CBD** allows for highly efficient single-step purification and immobilization on cellulose from a variety of sources, including regenerated cellulose beads or even cell wall cellulose from a transgenic plant expressing the recombinant **fusion protein**. Here, we present data on the purification of **CBDs** and **CBD-fusion proteins** from transgenic potato (Solanum tuberosum cv Desiree). The proteins were purified either directly from the recombinant expression system or from the plant exts. into which they were spiked. CBDclos can be purified directly on cellulose beads or, in the case of cell wall cellulose, 500 mg of CBDclos protein can be purified on 1 g wet weight plant material. In the case of CBDTma, the **CBD** binds to exogenously added cellulose and can be eluted with disaccharides such as glucose or cellobiose. Alternatively, the fusion partner can be cleaved and purified away from the **CBD**, if a protease cleavage site has been engineered into the **fusion protein**.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 155 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER:	2000:900778 CAPLUS
DOCUMENT NUMBER:	134:67153
TITLE:	Recombinant protein expression in plants as cellulose binding peptide **fusion protein** and isolation via affinity binding
INVENTOR(S):	Shani, Ziv; Shoseyov, Oded
PATENT ASSIGNEE(S):	CBD Technologies Ltd., Israel; Yissum Research Development Company of the Hebrew University of Jerusalem
SOURCE:	PCT Int. Appl., 87 pp. CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000077174	A1	20001221	WO 2000-IL330	20000607
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6331416	B1	20011218	US 1999-329234	19990610
CA 2376392	AA	20001221	CA 2000-2376392	20000607
AU 2000049475	A5	20010102	AU 2000-49475	20000607
EP 1185624	A1	20020313	EP 2000-931527	20000607
EP 1185624	B1	20041006		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2003502032	T2	20030121	JP 2001-503619	20000607
AT 278773	E	20041015	AT 2000-931527	20000607
PRIORITY APPLN. INFO.:			US 1999-329234 A	19990610
			WO 2000-IL330 W	20000607

AB A process of expressing a recombinant protein in a plant and of isolating the recombinant protein from the plant, is disclosed. The process consists of (a) providing a plant, a plant derived tissue or cultured plant cells expressing a **fusion protein** including the recombinant protein and a cellulose binding peptide being fused thereto, the **fusion protein** being compartmentalized within cells of the plant, plant derived tissue or cultured plant cells, so as to be sequestered from cell walls of the cells of the plant, plant derived tissue or cultured plant cells; (b) homogenizing the plant, plant derived tissue or cultured plant cells, so as to bring into contact the **fusion protein** with a cellulosic matter of the plant, plant derived tissue or cultured plant cells, to thereby effect affinity binding of the **fusion protein** via the cellulose binding peptide to the cellulosic matter, thereby obtaining a **fusion protein** cellulosic matter complex; and (c) isolating the **fusion protein** cellulosic matter complex. The recombinant protein can be released from the **fusion protein** by proteolysis at the unique protease recognition sequence. A vector for expression of such **fusion protein** is claimed. Cellulose Binding Domains (**CBDs**) are independently folding protein modules that bind strongly to different forms of cellulose via non-covalent hydrophobic interactions. We have engineered a variety of bifunctional protein fusions which bind to cellulose and retain the function of the fusion partner. These proteins can be expressed in standard systems including microbial, insect, mammalian and plant cells. The presence of the **CBD** allows for highly efficient single-step purification and immobilization on cellulose from a variety of sources, including regenerated cellulose beads or even cell wall cellulose from a transgenic plant expressing the recombinant **fusion protein**. Here, we present data on the purification of **CBDs** and **CBD-fusion proteins** from transgenic tobacco (Nicotiana tabaccum). The proteins were purified either directly from the recombinant expression system or from the plant exts. into which they were spiked. CBDclos can be purified directly on cellulose beads or, in the case of cell wall cellulose, 500 mg of CBDclos

protein can be purified on 1 g wet weight plant material. In the case of CBDTma, the **CBD** binds to exogenously added cellulose and can be eluted with disaccharides such as glucose or cellobiose. Alternatively, the fusion partner can be cleaved and purified away from the **CBD** , if a protease cleavage site has been engineered into the **fusion protein**.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 156 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:720308 CAPLUS
DOCUMENT NUMBER: 133:280644
TITLE: Process for partitioning of proteins
INVENTOR(S): Penttila, Merja; Nakari-Setala, Tiina; Fagerstrom, Richard; Selber, Klaus; Kula, Maria-regina; Linder, Markus; Tjerneld, Folke
PATENT ASSIGNEE(S): Valtion Teknillinen Tutkimuskeskus, Finland
SOURCE: PCT Int. Appl., 109 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000058342	A1	20001005	WO 2000-FI249	20000324
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2367826	AA	20001005	CA 2000-2367826	20000324
EP 1163260	A1	20011219	EP 2000-914217	20000324
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002543766	T2	20021224	JP 2000-608042	20000324
NZ 514891	A	20031031	NZ 2000-514891	20000324
AU 778477	B2	20041209	AU 2000-35621	20000324
NO 2001004534	A	20011126	NO 2001-4534	20010918
US 2006084164	A1	20060420	US 2005-252753	20051019
PRIORITY APPLN. INFO.:			FI 1999-667 A	19990325
			FI 1999-1782 A	19990820
			WO 2000-FI249 W	20000324
			US 2001-936823 A3	20011024

AB The present invention provides a method for isolation and purification of proteins in aqueous two-phase systems (ATPS). Specifically the invention provides processes for partitioning of proteins in ATPS by fusing the protein of interest to a targeting protein which has the ability of carrying the desired protein into one of the phases. Thus, the core of endoglucanase I (EGI)from Trichoderma reesii was produced in fed-batch fermns. as a **fusion protein** with the small protein hydrophobin I (HFBI). The fermentation broth was clarified by centrifugation, and the EGI-HFBI **fusion protein** was separated from the supernatant by ATPS using 2% (weight/weight) of the detergent C12-18-EO5. The purified **fusion protein** enriched in the top detergent phase was then removed by extraction with isobutanol.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 157 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:820530 CAPLUS
DOCUMENT NUMBER: 134:189817
TITLE: **Fusion proteins** containing cellulose-binding domains
AUTHOR(S): Park, Jae-Seon; Shin, Hae-Sun; Doi, Roy H.
CORPORATE SOURCE: Sampyo Foods Co., Ltd., Seoul, 132-040, S. Korea
SOURCE: Methods in Enzymology (2000), 326(Applications of Chimeric Genes and Hybrid Proteins, Pt. A), 418-429
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The construction of **fusion proteins** containing a cellulose-binding domain (**CBD**) is demonstrated and several potential uses of such **fusion proteins** for basic research and biotechnol. are described. **CBDs** are found as functional domains of many cellulolytic enzymes. The main role of **CBD** is to bind the enzyme or enzyme complex to cellulose and facilitate the interaction of the catalytic site with the substrate. **CBDs** are found in a wide variety of bacterial and fungal cellulases. (c) 2000 Academic Press.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 158 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:820529 CAPLUS
DOCUMENT NUMBER: 134:112519
TITLE: Fusions to self-splicing inteins for protein purification
AUTHOR(S): Xu, Ming-Qun; Paulus, Henry; Chong, Shaorong
CORPORATE SOURCE: New England Biolabs, Inc., Beverly, MA, 01915, USA
SOURCE: Methods in Enzymology (2000), 326(Applications of Chimeric Genes and Hybrid Proteins, Pt. A), 376-418
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein splicing involves the self-catalyzed excision of an intervening polypeptide segment, the intein, from a precursor protein, with the concomitant ligation of the flanking polypeptide sequence, the exteins, to yield a functional protein. The catalysis of protein splicing is entirely mediated by the intein and involves three distinct reaction steps. Elucidation of the sequence of steps that underlie protein splicing and studies on the effect of amino acid substitutions in the intein and adjacent residues on these steps led to the realization that catalysis of each of the steps in the protein splicing pathway is relatively independent and opened the way for modulating the protein splicing process as a protein engineering tool. It is described how inteins can be used to effect the self-catalyzed cleavage of **fusion proteins** at highly specific sites. Also, the mechanism of protein splicing in the context of the amino acid residues surrounding the splice junctions is briefly reviewed. (c) 2000 Academic Press.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 159 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:574661 CAPLUS
DOCUMENT NUMBER: 133:188065
TITLE: One step purification for recombinant human neurotrophic factor-3 with the splicing function of intein
AUTHOR(S): Yuan, Jing-Ming; Li, Zhuo-Yu; Wang, Ya-Mei; Xu,

Ming-Qun
CORPORATE SOURCE: Biotechnology Center, Shanxi University, Taiyuan, 030006, Peop. Rep. China
SOURCE: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (2000), 16(3), 335-339
CODEN: ZSHXF2; ISSN: 1007-7626
PUBLISHER: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Human neurotrophic factor-3 (hNT3) was amplified from human whole blood DNA by PCR and it was inserted into the multi-cloning sites of a special vector pTXB1, which contained the DNA fragment coding for intein-chitin binding domain (intein-**CBD**) to construct the recombinant plasmid pTXB-hNT3. After transferring plasmid pTXB-hNT3 into the host E. coli 2566, the strain was cultured in LB medium and induced by IPTG. The **fusion protein**, hNT3-Intein-**CBD**, was mainly expressed as the form of inclusion bodies. To make it soluble, the inclusion bodies were denatured in 8 M urea and renatured in the presence of 1.7 M urea containing GSH, GSSG and L-arginine. The soluble product possibly with its correct conformation was slowly loaded on a chitin beads column which was pre-equilibrated with 20 mmol/L Hepes buffer, pH 8.0, including 1.7 M urea and then was washed with the same buffer to harbor the **fusion protein** and remove the other proteins. After that, a solution of 50 mmol/L DTT in the same buffer was rapidly through the column and the cleavage reaction was carried out under intein splicing conditions for 48 h at 4°C or 25°C. Finally, hNT3 was eluated from the column with the washing buffer and examined on SDS-PAGE. The results showed that hNT3-intein-**CBD** could be partially cleaved by DTT and hNT3 was purified through one-step procedure on chitin beads column.

L3 ANSWER 160 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:691543 CAPLUS
DOCUMENT NUMBER: 135:4525
TITLE: Construction of a fusion expression vector including intein and the preliminary expression of human neurotrophic factor-3
AUTHOR(S): Fan, Zheng; Fan, Jun-Hu; Wang, Ru-Jing; Li, Zhuo-Yu
CORPORATE SOURCE: Biotechnol. Lab., Shanxi University, Taiyuan, 030006, Peop. Rep. China
SOURCE: Shanxi Daxue Xuebao, Ziran Kexueban (2000), 23(3), 250-253
CODEN: SDXKDT; ISSN: 0253-2395
PUBLISHER: Shanxi Daxue Xuebao Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB In order to construct a fusion expression vector which contains the protein splicing element intein, a modified vector, pExSec I/Linder, was made from inserting a 49-bp polynucleotide with several endonuclear cleavage sites into the site of EcoR I/BamH I in MCS of pExSec I. Then the new expression vector, pExIC, was obtained with inserting the intein-**CBD** fragment from pMYB129 into pExSec I/Linder. Meanwhile, the recombinant plasmid pExIC-hNT-3 was constructed when hNT-3 gene which was amplified with human blood DNA as the template by PCR was inserted which was amplified with human blood DNA as the template by PCR was inserted into pExIC. After transformation, the strain Escherichia coli BL21/pExIC-hNT-3 was cultured in LB media and the **fusion protein**, intein-hNT-3 was expressed after IPTG induction. According to the splicing principle of intein, the target protein, hNT-3 was obtained with DTT excision on SDS-PAGE.

L3 ANSWER 161 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2000:779465 CAPLUS
DOCUMENT NUMBER: 134:38967
TITLE: Phage display of cellulose binding domains for biotechnological application
AUTHOR(S): Benhar, Itai; Tamarkin, Aviva; Marash, Lea; Berdichevsky, Yevgeny; Yaron, Sima; Shoham, Yuval; Lamed, Raphael; Bayer, Edward A.
CORPORATE SOURCE: Department of Molecular Microbiology and Biotechnology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Israel
SOURCE: ACS Symposium Series (2000), 769(Glycosyl Hydrolases for Biomass Conversion), 168-189
CODEN: ACSMC8; ISSN: 0097-6156
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 65 refs. In recent years, cellulose-binding domains (**CBDs**) derived from the cellulolytic systems of cellulose-degrading microorganisms have become a focal point of attention for a wide range of biotechnol. applications. The low cost and availability of cellulose matrixes have rendered **CBDs** attractive as affinity tags for the purification and immobilization of a plethora of proteins. Intensive studies of cellulose degradation pathways and the identification of components of the cellulose-degrading machinery have contributed significantly to our understanding of the structure and function of **CBDs**. The time is now ripe to utilize engineered **CBDs** to improve existing applications and to devise novel ones. Here we describe our recent results of expts. where the Clostridium thermocellum scaffoldin **CBD** was genetically engineered for such purposes. We describe the development of a novel phage display system where the C. thermocellum **CBD** is displayed as a **fusion protein** with single-chain antibodies. Our system is a filamentous (M13) phage display system that enables the efficient isolation and.
REFERENCE COUNT: 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 162 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:710413 CAPLUS
DOCUMENT NUMBER: 134:38648
TITLE: The structure and function of cellulose-binding domain of cellulase
AUTHOR(S): Wang, Tianhong; Wang, Chunhui; Gao, Peiji
CORPORATE SOURCE: The State Key Laboratory of Microbial Technology, Shandong University, Jinan, 250100, Peop. Rep. China
SOURCE: Shengwu Gongcheng Jinzhan (2000), 20(2), 37-40
CODEN: SGJHA2; ISSN: 1003-3505
PUBLISHER: Zhongguo Kexueyuan Wenxian Qingbao Zhongxin
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Chinese
AB A discussion and review with 20 refs. Most cellulases consist of catalytic domains and cellulose binding domains(**CBDs**), which can bind to cellulose and are conserved in some amino acid sequences. Cellulose binding domains improve the binding and facilitate the activity of catalytic domains on the insol. substrate, but not on soluble substrate. The results of investigations on structure and function, and subsequent mutagenesis of the **CBDs** indicated that **CBDs** rely on several aromatic amino acids for binding to the cellulose surfaces. Some experiment results showed that **CBDs** of exoglucanases are able to disrupt the crystalline cellulose, facilitate the enzymic degradation of cellulose.
The structural domain as **CBDs** has been successfully used in purification and immobilization of numerous examples of **fusion proteins**. The improved understanding of the structure and

function of **CBDs** are significant to understanding of enzymic functionary mechanism, and the development of cellulase biotechnol.

L3 ANSWER 163 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:327239 CAPLUS
TITLE: Recombinant protein purification using **CBDs**.
AUTHOR(S): Shpigel, E.; Goldlust, A.; Eshel, A.; Shoseyov, O.; Kilburn, D. G.; Gilkes, N.; Guarna, M. M.; Kwan, E. M.; Boraston, A. B.; Warren, R. A. J.
CORPORATE SOURCE: CBD-Technologies Ltd, Rehovot, 76100, Israel
SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), BIOT-020. American Chemical Society: Washington, D. C. CODEN: 69CLAC
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Cellulose Binding Domains (**CBDs**) are independently folding protein modules that bind strongly to different forms of cellulose via non-covalent hydrophobic interactions. We have engineered a variety of bifunctional protein fusions which bind to cellulose and retain the function of the fusion partner. These proteins can be expressed in standard systems including microbial, insect, mammalian and plant cells. The presence of the **CBD** allows for highly efficient single-step purification and immobilization on cellulose from a variety of sources, including regenerated cellulose beads or even cell wall cellulose from a transgenic plant expressing the recombinant **fusion protein**. Here, we present data on the purification of **CBDs** and **CBD-fusion proteins** on regenerated cellulose and potato and corn cell wall exts. and filtrates. The proteins were purified either directly from the recombinant expression system or from the plant exts. into which they were spiked. CBDclos can be purified directly on cellulose beads or, in the case of cell wall cellulose, 500 mg of CBDclos protein can be purified on 1 g wet weight plant material. **CBDs** and **CBD-fusion proteins** which bind poorly to plant cell wall cellulose, can be purified from the plant filtrate by addition of exogenous cellulose beads or powder. In the case of CBDTma, the **CBD** binds to exogenously added cellulose and can be eluted with disaccharides such as glucose or cellobiose. Immobilized **CBD-fusion proteins** may be used in biosepn. applications. Alternatively, the fusion partner can be cleaved and purified away from the **CBD**, if a cleavage site has been engineered into the **fusion protein**. We have inserted the cleavage recognition site (IEGR) for the coagulation pathway enzyme Factor X into the region separating maltose binding protein from its calbindin **fusion protein** (MBP-calbindin) and demonstrated that an immobilized **CBD**-Factor X enzyme can be used to cleave this **fusion protein**. Results indicate that the enzyme is efficient (cleavage of 97% or 86 % of substrate in 24 h at an enzyme: substrate ratio of 1:200 or 1:5,000, resp.). The immobilized enzyme appears to be quite stable over time with only a 25% loss of activity after 20 days of continuous operation.

L3 ANSWER 164 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:283852 CAPLUS
DOCUMENT NUMBER: 134:142494
TITLE: Fusion expression of human neurotrophic factor-3 with intein by temperature induction
AUTHOR(S): Li, Zhuoyu; Fan, Junhu; Wang, Juxiang; Yuan, Jingming
CORPORATE SOURCE: Biotechnol. Laboratory, Shanxi University, Taiyuan, 030006, Peop. Rep. China
SOURCE: Gaojishu Tongxun (2000), 10(3), 1-4 CODEN: GTONE8; ISSN: 1002-0470
PUBLISHER: Gaojishu Tongxun Zazhishe

DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB A new recombinant plasmid (pLZY01) containing a fusion gene encoding human neurotrophic factor-3 and the chitin binding domain of intein (hNT3-intein-**CBD**) was cloned into Escherichia coli. The E. coli BL21/pLZY01 strain was cultured in LBbB + Amp medium to A600 = 0.8 at 37°, and then incubated at 42° for 3 h. A 42 kD **fusion protein** was obtained. There were 3 bands at 42 kD (hNT3-intein-**CBD**), 27 kD (intein- **CBD**) and 15 kD (hNT3) on SDS-PAGE after DTT incomplete reduction of the 42 kD **fusion protein**. A 15 kD pure product (hNT3) was obtained after denaturation and renaturation of inclusion bodies through affinity chromatog. on chitin beads and DTT cleavage. Using an assay with the dorsal root ganglion of chicken embryo, both **fusion protein** and target protein (hNT3) may have the biol. activities at the concentration of 80 ng and 25 ng, resp.

L3 ANSWER 165 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:595392 CAPLUS
DOCUMENT NUMBER: 131:224476
TITLE: Chitinase chitin-binding fragments and their use in chitin determination and as anti-fungal agents
INVENTOR(S): Gray, Patrick W.; Tjoelker, Larry W.
PATENT ASSIGNEE(S): Icos Corporation, USA
SOURCE: PCT Int. Appl., 83 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9946390	A1	19990916	WO 1999-US5343	19990312
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6200951	B1	20010313	US 1998-39198	19980312
CA 2323070	AA	19990916	CA 1999-2323070	19990312
AU 9929989	A1	19990927	AU 1999-29989	19990312
AU 763582	B2	20030724		
BR 9908724	A	20001121	BR 1999-8724	19990312
EP 1078073	A1	20010228	EP 1999-911320	19990312
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002505882	T2	20020226	JP 2000-535757	19990312
NO 2000004522	A	20001101	NO 2000-4522	20000911
PRIORITY APPLN. INFO.:			US 1998-39198 A	19980312
			WO 1999-US5343 W	19990312

AB The present invention provides chitin-binding fragments of human chitinase, polynucleotide sequences encoding such fragments, and materials and methods for the recombinant production of human chitinase fragment products which are expected to be useful in products for detecting chitin, binding chitin, and treating fungal infections or for development of products useful for treating the same. Thus, human chitinase cDNA was cloned and sequenced. The chitinase gene was most strongly expressed in lung and ovary. The C-terminal 72 amino acids were required for

chitin-binding activity but not for hydrolysis of triacetylchitotriose. Chitin-binding fragments were produced with recombinant yeast. These fragments may be useful in treating such fungal diseases as Aspergillosis, Candidiasis, endophthalmitis, endocarditis, etc.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 166 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:359641 CAPLUS
DOCUMENT NUMBER: 131:29287
TITLE: Pectate lyases from Bacillus species suitable for industrial processes
INVENTOR(S): Andersen, Lene Nonboe; Schulein, Martin; Lange, Niels Erik Krebs; Bjornvad, Mads Eskelund; Moller, Soren; Glad, Sanne O. Schroder; Kauppinen, Markus Sakari; Schnorr, Kirk; Kongsbaek, Lars
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 97 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9927084	A1	19990603	WO 1998-DK515	19981124
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6124127	A	20000926	US 1998-73684	19980506
US 6258590	B1	20010710	US 1998-184217	19981102
CA 2310562	AA	19990603	CA 1998-2310562	19981124
AU 9914825	A1	19990615	AU 1999-14825	19981124
EP 1032658	A1	20000906	EP 1998-958820	19981124
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
BR 9815007	A	20001003	BR 1998-15007	19981124
TR 200001489	T2	20001121	TR 2000-200001489	19981124
US 6187580	B1	20010213	US 1998-198955	19981124
JP 2001526022	T2	20011218	JP 2000-522226	19981124
CA 2348447	AA	20000511	CA 1999-2348447	19991027
WO 2000026464	A2	20000511	WO 1999-US24489	19991027
WO 2000026464	A3	20000810		
W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
BR 9914968	A	20010710	BR 1999-14968	19991027
TR 200101217	T2	20011022	TR 2001-200101217	19991027
EP 1159479	A2	20011205	EP 1999-960137	19991027
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002529610	T2	20020910	JP 2000-579830	19991027
US 6368843	B1	20020409	US 2000-694531	20001023
US 2002115194	A1	20020822	US 2001-789266	20010220

US 6630342	B2	20031007		
US 2002142438	A1	20021003	US 2002-72152	20020207
US 6677147	B2	20040113		
US 2004067572	A1	20040408	US 2003-655433	20030904

PRIORITY APPLN. INFO.:

DK 1997-1343	A	19971124
DK 1997-1344	A	19971124
US 1998-73684	A	19980506
US 1998-184217	A	19981102
US 1997-67240P	P	19971202
US 1997-67249P	P	19971202
US 1998-198955	A1	19981124
WO 1998-DK515	W	19981124
WO 1999-US24489	W	19991027
US 2000-694531	A1	20001023
US 2002-72152	A1	20020207

AB A novel group of pectate lyases comprising the amino acid sequence Asn-Leu-Asn-Ser-Arg-Val-Pro (NLNSRVP) belonging to Family 1 of polysaccharide lyases have good performance in industrial processes under neutral or alkaline conditions such as laundering and textile processing. The pectate lyase may be derivable from Bacillus species. Sequences claimed in the patent were not available in the document.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 167 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1999:359640 CAPLUS
DOCUMENT NUMBER: 131:15715
TITLE: Pectin-degrading enzymes from Bacillus licheniformis and their industrial applications
INVENTOR(S): Andersen, Lene Nonboe; Schulein, Martin; Lange, Niels Erik Krebs; Bjornvad, Mads Eskelund; Schnorr, Kirk
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9927083	A1	19990603	WO 1998-DK514	19981124
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6124127	A	20000926	US 1998-73684	19980506
CA 2310806	AA	19990603	CA 1998-2310806	19981124
AU 9914339	A1	19990615	AU 1999-14339	19981124
EP 1032657	A1	20000906	EP 1998-958214	19981124
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
TR 200001494	T2	20000921	TR 2000-200001494	19981124
BR 9815015	A	20001003	BR 1998-15015	19981124
US 6187580	B1	20010213	US 1998-198955	19981124
JP 2001524310	T2	20011204	JP 2000-522225	19981124
US 6368843	B1	20020409	US 2000-694531	20001023
US 2002142438	A1	20021003	US 2002-72152	20020207
US 6677147	B2	20040113		
US 2004067572	A1	20040408	US 2003-655433	20030904

PRIORITY APPLN. INFO.:

DK 1997-1344	A	19971124
US 1998-73684	A	19980506
DK 1997-1343	A	19971124
US 1997-67240P	P	19971202
US 1997-67249P	P	19971202
US 1998-184217	A2	19981102
US 1998-198955	A1	19981124
WO 1998-DK514	W	19981124
US 2000-694531	A1	20001023
US 2002-72152	A1	20020207

AB Pectin-degrading enzymes derived from or endogenous to Bacillus licheniformis or other Bacillus species which are ≥99% homologous to Bacillus Licheniformis based on aligned 16S rDNA sequences have optimum activity at pH higher than 8. The pectin-degrading enzymes belong to the enzyme classes pectate lyases (EC 4.2.2.2), pectin lyases (EC 4.2.2.10), and polygalacturonases (EC 3.2.1.15) and are useful in industrial processes under alkaline conditions such as in textile processing and as an active ingredient, e.g. in laundry detergents and hard surface cleaning products. Gene and deduced amino acid sequences are provided for two pectate lyases, a pectin lyase, and a polygalacturonase.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 168 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1999:581656 CAPLUS

DOCUMENT NUMBER: 132:19316

TITLE: Construction and use of low-copy number T7 expression vectors for purification of problem proteins: purification of Mycobacterium tuberculosis RmlD and Pseudomonas aeruginosa LasI and RhlI proteins, and functional analysis of purified RhlI

AUTHOR(S): Hoang, T. T.; Ma, Y.; Stern, R. J.; McNeil, M. R.; Schweizer, H. P.

CORPORATE SOURCE: Department of Microbiology, Colorado State University, Fort Collins, CO, USA

SOURCE: Gene (1999), 237(2), 361-371
CODEN: GENED6; ISSN: 0378-1119

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Purification of proteins from Escherichia coli under native conditions is often hampered by inclusion-body formation after overexpression from T7 promoter-based expression vectors. This is probably due to the relatively high copy number of the ColE1-based expression vectors. To circumvent these problems, the low-copy-number pViet and pNam expression vectors were constructed. These vectors contain the pSC101 origin of replication and allow the expression of oligohistidine and intein chitin-binding domain **fusion proteins**, resp. Since pViet and pNam do not replicate in E. coli B strains, an E. coli K-12 host strain [SA1503(DE3)] was constructed. This strain is defective in the Lon and OmpT proteases and allows IPTG-inducible expression of recombinant proteins from the T7 promoter. The new vectors were successfully tested by purification of three very insol. proteins (RmlD, LasI and RhlI) under non-denaturing conditions, and all three proteins retained enzymic activity. The purified hexahistidine (His6)-tagged Pseudomonas aeruginosa RhlI protein was subjected to more detailed analyses, which indicated that (1) only butyryl-acyl carrier protein (ACP) and S-adenosylmethionine (SAM) were required for synthesis of N-butyryl-l-homoserine lactone; (2) when present at physiol. concns., butyryl-CoA and NADPH were not substrates for RhlI; (3) RhlI was able to synthesize N-hexanoyl-l-homoserine lactone from hexanoyl-ACP and SAM; (4) RhlI was able to direct synthesis of N-butyryl-l-homoserine lactone from crotonyl-ACP in a reaction coupled to purified P. aeruginosa FabI (enoyl-ACP reductase).

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 169 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:144081 CAPLUS
TITLE: Detoxification of organophosphates by recombinant E. coli with co-expression of cellulose binding domain and organophosphate hydrolase on the cell surface
AUTHOR(S): Chen, Wilfred; Wang, Aijun A.; Mulchandani, Ashok
CORPORATE SOURCE: Dept. of Chemical and Environmental Engineering, University of California, Riverside, CA, 92521, USA
SOURCE: Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25 (1999), BIOT-067. American Chemical Society: Washington, D. C. CODEN: 67GHA6
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB The Cellulose Binding Domain from Cellulomonas fimi exoglucocase (**CBD**) was coexpressed with organophosphorus hydrolase (OPH) on the surface of Escherichia coli using an Lpp-OmpA fusion system. Production of the **CBD-fusion protein** was verified by immunoblotting membrane fractions with **CBD** antisera. Protease accessibility expts. demonstrated that both **CBD** and OPH were anchored on the outer surface of the cell membrane. Cultures expressing both Lpp-OmpA-**CBD** and Lpp-OmpA-OPH were shown to be capable of binding to a variety of cellulose materials while simultaneously degrading organophosphates such as paraoxon. The novel biocatalysts may prove useful for large scale detoxification of organophosphate pesticides, enabling simple and efficient immobilization.

L3 ANSWER 170 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:293601 CAPLUS
DOCUMENT NUMBER: 128:318028
TITLE: Isolation, cloning, and extracellular expression of cellulose-binding proteins genes in Bacillus, other bacteria, and fungi.
INVENTOR(S): Bjornvad, Mads Eskelund; Schulein, Martin; Jorgensen, Per Lina
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.; Bjornvad, Mads Eskelund; Schulein, Martin; Jorgensen, Per Lina
SOURCE: PCT Int. Appl., 41 pp. CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9818905	A1	19980507	WO 1997-DK477	19971028
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9746997	A1	19980522	AU 1997-46997	19971028
CN 1235635	A	19991117	CN 1997-199193	19971028
EP 975745	A1	20000202	EP 1997-909214	19971028
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
US 6060274	A	20000509	US 1997-959212	19971028

JP 2001507569 T2 20010612 JP 1998-519936 19971028
PRIORITY APPLN. INFO.: DK 1996-1192 A 19961028
DK 1996-1426 A 19961213
WO 1997-DK477 W 19971028

AB A Bacillus host transformed with a vector comprising a DNA sequence encoding for a cellulose-binding domain (**CBD**) and capable of expressing said sequence, the expressed polypeptide protein consisting essentially of one or more non-catalytical domains; the cellulose-binding domain having a mol. weight in the range of from 4-35 kilodaltons and being obtainable from a microorganism or from a plant, preferably from a bacterium or a fungus; the Bacillus host e.g. being one of the species (Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus stearothermophilus) and Bacillus amyloliquefaciens; and a Bacillus expression vector carrying an inserted DNA sequence encoding for a cellulose binding domain; and a method for producing a cellulose binding domain polypeptide in a Bacillus host cell. Thus, Bacillus genes for cellulose-binding proteins, and genes encoding chimeric proteins with similar domains were isolated. These genes were inserted into expression vectors and expressed in Escherichia coli as well as Bacillus host cells. Bacterial or fungal cellulose-binding proteins are claimed. The cellulose-binding protein genes and bacterial hosts include Butyrivibrio, Cellulomonas, Clostridium, Microbispora, Micromonospora, Pseudomonas, Streptomyces, Thermomonospora, Caldocellum, Erwinia, Myxococcus, Cellvibrio, Thermoanaerobacterium, and Thermotoga. Fungal sources include Agaricus, Dictyostelium, Fusarium, Humicola, Neocallimastix, Neurospora, Limulus, Penicillium, Phanerochaete, and Trichoderma. Bacterial or fungal cellulose-binding proteins are claimed. Green fluorescent protein is used as a reporter chemical compound in these expression vectors.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 171 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:67803 CAPLUS
DOCUMENT NUMBER: 130:293563
TITLE: Purification of a **fusion protein** using the family VI cellulose-binding domain of Clostridium stercorarium XynA
AUTHOR(S): Sakka, Kazuo; Karita, Shuichi; Kimura, Tetsuya; Ohmiya, Kunio
CORPORATE SOURCE: Faculty of Bioresources, Mie University, Tsu, 514, Japan
SOURCE: Annals of the New York Academy of Sciences (1998), 864(Enzyme Engineering XIV), 485-488
CODEN: ANYAA9; ISSN: 0077-8923
PUBLISHER: New York Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In this study, the usefulness of the family VI cellulose-binding domain(**CBD**) as an affinity tag is evaluated. It is found the VI **CBD** as affinity tag is advantageous for the rapid purification of unstable proteins such as endoglucanase IV in addition to stable proteins. However, some improvement is necessary to shorten the purification period.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 172 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:230343 CAPLUS
TITLE: Characterization of cellulose binding domain cytokine **fusion proteins** for culture of cytokine dependent cells on cellulose surfaces.
AUTHOR(S): Jervis, E. J.; Doheny, J. G.; Guarna, M.; Kilburn, D. G.
CORPORATE SOURCE: Biotechnology Laboratory, University British Columbia,

Vancouver, BC, V6T 1Z3, Can.
SOURCE: Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17 (1997), BIOC-277. American Chemical Society: Washington, D. C.
CODEN: 64AOAA
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB We have produced **fusion proteins** linking the cellulose binding domain (**CBD**) of a bacterial cellulase to murine stem cell growth factor (SCF). This hybrid protein retains the properties of both fusion partners. The application of **CBDs** for immobilization of SCF for the culture of primary bone marrow cells is demonstrated. **CBD**-SCF presented on cellulose polarizes receptors to the cell / cellulose interface and permits patching and activation of receptors in the cell membrane. Western blot anal. and confocal fluorescence microscopy revealed that binding **CBD**-SCF to cellulose increases the duration of SCF receptor phosphorylation and may slow turnover of activated receptor. The proliferative activity of **CBD**-SCF bound on various cellulosic materials will be presented.

L3 ANSWER 173 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:506431 CAPLUS
DOCUMENT NUMBER: 131:319378
TITLE: Characterization of cellulose-binding domains from Clostridium stercorarium xylanase XynA and their application as an affinity-tag for rapid purification of **fusion proteins**
AUTHOR(S): Sakka, Kazuo; Kimura, Tetsuya; Karita, Shuichi; Ohmiya, Kunio
CORPORATE SOURCE: Faculty of Bioresources, Mie University, Tsu, 514, Japan
SOURCE: Recent Research Developments in Agricultural & Biological Chemistry (1997), 1, 243-248
CODEN: RRACFD
PUBLISHER: Research Signpost
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two cellulose-binding domains belonging to family VI were identified in Clostridium stercorarium xylanase XynA and characterized. Deletion anal. indicated that each of the **CBDs** arranged in tandem, CBDI and CBDII, functions as a **CBD** independently. XynA bound to amorphous cellulose but not highly crystalline cellulose, and it was released from the cellulose-protein complex by wash with a cellobiose solution This property was applicable to construct an efficient affinity purification system by constructing a **fusion protein** comprising a target polypeptide and the **CBDs**. A chimeric protein composed of a Ruminococcus albus endoglucanase EGIV and CBDII was easily purified from cell-free extract by its adsorption to cellulose and desorption by cellobiose. Artificial connection of the **CBDs** confirmed an enhanced activity against insol. cellulose on EGIV.
REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 174 OF 224 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:230109 CAPLUS
TITLE: Cellulose binding domains diffuse in 2-D when bound to cellulose.
AUTHOR(S): Jervis, E. J.; Haynes, C. A.; Kilburn, D. G.
CORPORATE SOURCE: Department Chemical Engineering, University British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17 (1997), BIOC-044. American Chemical Society: Washington, D. C.